

GERMINATION OF WEED SEEDS: II. THE INFLUENCE OF TILLAGE TREATMENTS ON GERMINATION<sup>1</sup>W. S. CHEPIL<sup>2</sup>*Soil Research Laboratory, Swift Current, Saskatchewan*

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The general farm practice in the prairie region of western Canada consists of one or two spring grain crops, which are usually wheat, alternated with one year of fallow. The purpose of the fallow is primarily to conserve moisture for the following year's crop and incidentally to reduce weed infestations. The number of cultivations on fallow range from 1 to 4, and in some cases even more if perennial weeds are to be eradicated. One cultivation is usually sufficient in preparing land for seeding.

The tillage implements that are used produce various effects on the surface soil. Some implements, such as the plow, turn the soil completely over, whereas others, such as the disc harrow, essentially cut and stir the first few inches of the surface soil. Still others, such as the blade weeder, destroy the weeds by severing their root system but leave the soil in essentially the same position as before. The different types of tillage implements might be expected to have various effects on the behaviour of weed seeds in the soil. It was therefore decided to determine the extent of germination and longevity of weed seeds under various conditions, such as are generally produced by the most common tillage practices in this region, and to determine what type of tillage practices are the most suitable from the standpoint of weed control. The study was undertaken to find the influence of the following specific factors:

- (1) Depth at which weed seeds germinate most readily.
- (2) Methods of cultivation in relation to germination and growth of weeds.
- (3) Packing as it affects germination and vitality of weed seeds.
- (4) Soil moisture in relation to delayed germination, or dormancy.
- (5) Time of the year at which weed seeds germinate most readily.
- (6) Mortality of weed seeds under different tillage treatments.
- (7) Soil type in relation to germination and growth of weeds.

## MATERIALS AND METHODS

Seeds of Russian thistle (*Salsola Pestifer*), stinkweed (*Thlaspi arvense*), wild mustard (*Sinapis arvensis*), tumbling mustard (*Sisymbrium altissimum*) and red-root pigweed (*Amaranthus retroflexus*) were chosen at random from samples grown during the current year and containing plump, well-matured

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kernels. Duplicate lots of one thousand seeds were placed in the field about November 15 in open bottom galvanized iron trays 12 inches long and 6 inches wide, containing sterilized soil to the full depth of 8 inches.

Simulated cultural treatments were carried out on 3 soil types, Haverhill loam, Hatton fine sandy loam, and Sceptre heavy clay, as follows:

- (1) Seeds scattered on the surface, no tillage.
- (2) Seeds mixed into the upper 2.5-inch layer of soil, no subsequent tillage.
- (3) Seeds mixed into the upper 2.5-inch layer of soil, cultivated to 2.5-inch depth at intervals of about one month during the growing season.
- (4) Seeds mixed into the upper 2.5-inch layer of soil, periodically cultivated as in (3) and packed after each cultivation.
- (5) Seeds mixed into the upper 2.5-inch layer of soil, periodically cultivated as in (3) and water added to keep the soil continually moist.
- (6) Seeds mixed into the upper 6-inch layer of soil, no subsequent tillage.
- (7) Seeds mixed into the upper 6-inch layer of soil, plowed to 6-inch depth in June, cultivated periodically to 2.5-inch depth during the rest of the season.

Cultivation was simulated by a moderate amount of stirring of the soil to a 2.5-inch depth and plowing was simulated by turning the 6-inch layer of surface soil completely over. Packing was done with the use of a 4-inch diameter metal roller, with pressure applied on the roller at about 5 pounds per linear inch.

To avoid contamination, the experiment was carried out in an area surrounded by sod. The trays were covered with window screening to prevent damage from rodents and insects.

No seedlings appeared immediately after November 15, but records of seedlings emerging during the next season were made as soon as they appeared, when they were identified, counted, and pulled out with the roots.

In the fall, after the seeds had been left in the soil for one year, the content of each tray were removed and a fresh lot of similar soil and weed seeds were put in to repeat the experiment for the following year. The soils removed were washed through a 60-mesh sieve, which was fine enough to prevent the loss of even the smallest seeds, and the residues along with the seeds that survived the field treatment were placed in shallow saucers in a layer not exceeding 1 inch in depth and subjected to repeated germination tests in the laboratory with occasional stirring until no more seeds would germinate. Virtually all viable seeds germinated within a period of 3 years, although for some of the earlier experiments tests were continued for a period of 5 years.

## RESULTS

By far the highest percentage emergence of seedlings of the species studied was from seeds lying on the surface of the ground (Table 1). With the exception of Russian thistle, the high emergence from this treatment



TABLE 1.—THE INFLUENCE OF TILLAGE TREATMENTS ON GERMINATION AND DORMANCY OF WEED SEEDS IN THE FIELD

| Weed            | Soil       | Treat-<br>ment<br>* | Seasonal<br>emergence<br>(5-year average) |                  | Viable seeds left in the soil after<br>exposure during† |               |               |               |               |              |
|-----------------|------------|---------------------|---|------------------|---|---------------|---------------|---------------|---------------|--------------|
|                 |            |                     | To<br>June 30                             | After<br>June 30 | 1936-<br>1937   | 1937-<br>1939 | 1939-<br>1940 | 1940-<br>1941 | 1941-<br>1942 | Aver-<br>age |
|                 |            |                     | %   | %                | %   | %             | %             | %             | %             | %            |
| Russian thistle | Loam       | 1                   | 42.6                                      | 1.3              | 0.3   | 0             | 0             | 0             | 0.1           | 0.1          |
|                 |            | 2                   | 22.2                                      | 0.8              | 0.1   | 0             | 0.1           | 0.1           | 0.2           | 0.1          |
|                 |            | 3                   | 21.9                                      | 0.6              | 0.1   | 0             | 0.1           | 0.25          | 0.2           | 0.1          |
|                 |            | 4                   | 20.9                                      | 0.5              | 0.1   | 0             | 0.2           | 0.1           | 0.1           | 0.1          |
|                 |            | 5                   | 25.3                                      | 0.1              | 0.1   | 0             | 0.3           | 0.15          | 0.3           | 0.2          |
|                 |            | 6                   | 11.1                                      | 0.4              | **  | 0             | 1.3           | 0.15          | 0.6           | 0.5          |
|                 |            | 7                   | 11.7                                      | 0.4              | **  | 0             | 0.7           | 0.15          | 0.1           | 0.2          |
|                 | Sandy loam | 2                   | 18.1                                      | 0.2              | 0   | 0             | 0.2           | 0.25          | 0.2           | 0.1          |
|                 |            | 3                   | 16.7                                      | 0.2              | 0.1   | 0             | 0.7           | 0.1           | 0             | 0.2          |
|                 |            | 4                   | 18.1                                      | 0.1              | 0.1   | 0             | 0.9           | 0.4           | 0             | 0.3          |
|                 |            | 7                   | 7.5                                       | 0.5              | **  | 0             | 0.6           | 0.4           | 0.2           | 0.3          |
|                 | Clay       | 2                   | 35.4                                      | 0.7              | 1.5   | 0             | 0.5           | 0.2           | 0.2           | 0.5          |
|                 |            | 3                   | 32.3                                      | 1.1              | 3.3   | 0             | 0.5           | 0.1           | 0.2           | 0.8          |
|                 |            | 4                   | 40.3                                      | 1.3              | 3.9   | 0             | 0.2           | 0.2           | 0.5           | 0.9          |
|                 |            | 7                   | 19.4                                      | 1.8              | 6.2   | 0             | 1.9           | 0.15          | 0.4           | 1.7          |
| Stinkweed       | Loam       | 1                   | 45.5                                      | 8.7              | 0.2   | 0.2           | 3.7           | 0.5           | 0.6           | 1.0          |
|                 |            | 2                   | 27.0                                      | 1.7              | 7.7   | 0.9           | 25.6          | 13.4          | 1.6           | 9.8          |
|                 |            | 3                   | 27.4                                      | 8.3              | 3.0   | 0             | 13.1          | 8.1           | 1.8           | 5.2          |
|                 |            | 4                   | 31.2                                      | 8.6              | 6.8   | 0.2           | 26.6          | 7.8           | 0.9           | 8.5          |
|                 |            | 5                   | 33.2                                      | 8.5              | 5.6   | 0             | 26.9          | 8.6           | 1.4           | 8.5          |
|                 |            | 6                   | 8.2                                       | 1.0              | 7.2   | 9.8           | 55.1          | 9.2           | 44.5          | 25.2         |
|                 |            | 7                   | 18.4                                      | 5.4              | **  | 2.2           | 22.6          | 13.5          | 25.0          | 15.8         |
|                 | Sandy loam | 2                   | 27.8                                      | 1.6              | **  | 0.7           | 43.5          | 10.5          | 3.8           | 14.6         |
|                 |            | 3                   | 24.4                                      | 8.4              | 1.5   | 0.6           | 27.2          | 5.1           | 4.0           | 7.7          |
|                 |            | 4                   | 28.3                                      | 8.3              | 4.6   | 0.4           | 20.4          | 6.0           | 4.0           | 7.1          |
|                 |            | 7                   | 11.0                                      | 7.1              | **  | 1.9           | 16.6          | 14.3          | 20.3          | 13.3         |
|                 | Clay       | 2                   | 30.5                                      | 3.2              | 15.1  | 9.6           | 46.8          | 32.2          | 14.2          | 23.6         |
|                 |            | 3                   | 32.5                                      | 13.4             | 6.2   | 10.1          | 44.0          | 10.0          | 14.5          | 17.0         |
|                 |            | 4                   | 36.6                                      | 12.0             | 9.3   | 6.7           | 31.1          | 11.9          | 9.9           | 13.8         |
|                 |            | 7                   | 18.7                                      | 12.7             | 23.4  | 7.8           | 38.4          | 20.8          | 28.6          | 23.8         |
| Wild mustard    | Loam       | 1                   | 48.6                                      | 8.8              | Not included in the experiment                          | 0             | 2.2           | 1.6           | 0.6           | 1.1          |
|                 |            | 2                   | 39.4                                      | 2.1              |   | 3.1           | 5.6           | 20.0          | 8.6           | 9.3          |
|                 |            | 3                   | 41.1                                      | 6.0              |   | 0.2           | 3.3           | 7.3           | 3.8           | 3.6          |
|                 |            | 4                   | 37.1                                      | 5.7              |   | 1.6           | 4.8           | 4.6           | 5.6           | 4.2          |
|                 |            | 5                   | 45.0                                      | 3.1              |   | 1.6           | 2.6           | 6.6           | 3.0           | 3.4          |
|                 |            | 6                   | 14.5                                      | 1.1              |   | 9.8           | 3.2           | 30.0          | 23.0          | 16.5         |
|                 |            | 7                   | 20.3                                      | 3.6              |   | 3.6           | 3.3           | 17.8          | 7.4           | 8.0          |
|                 | Sandy loam | 2                   | 20.0                                      | 1.7              |   | 2.1           | 6.3           | 9.6           | 7.6           | 4.9          |
|                 |            | 3                   | 22.0                                      | 4.2              |   | 1.3           | 3.8           | 6.8           | 7.8           | 4.9          |
|                 |            | 4                   | 28.8                                      | 4.7              |   | 0.7           | 2.5           | 8.4           | 6.8           | 4.6          |
|                 |            | 7                   | 13.9                                      | 5.2              |   | 8.5           | 22.8          | 21.6          | 20.4          | 18.3         |
|                 | Clay       | 2                   | 43.0                                      | 2.9              |   | 2.1           | 3.9           | 10.6          | 2.9           | 4.9          |
|                 |            | 3                   | 48.5                                      | 6.1              |   | 1.2           | 3.3           | 5.1           | 1.5           | 2.8          |
|                 |            | 4                   | 47.6                                      | 5.8              |   | 2.6           | 3.0           | 6.8           | 1.0           | 3.4          |
|                 |            | 7                   | 26.1                                      | 6.0              |   | 4.0           | 11.4          | 16.4          | 7.6           | 9.8          |

\* As indicated on page 355.

\*\* Samples were ruined accidentally before it was possible to complete the determination.

† That is, from Oct. 31 until the following Oct. 31, except for the 1937 to 1939 period when treatment continued for 2 years.

‡ The 1936-1937 partial results are not included in the average.

TABLE 1.—THE INFLUENCE OF TILLAGE TREATMENTS ON GERMINATION AND DORMANCY OF WEED SEEDS IN THE FIELD—*Concluded*

| Weed             | Soil       | Treatment | Seasonal emergence<br>(5-year average) |                  | Viable seeds left in the soil after exposure during. |               |               |               |               |         |
|------------------|------------|-----------|--|------------------|--|---------------|---------------|---------------|---------------|---------|
|                  |            |           | To<br>June 30                          | After<br>June 30 | 1936-<br>1937  | 1937-<br>1939 | 1939-<br>1940 | 1940-<br>1941 | 1941-<br>1942 | Average |
| Tumbling Mustard | Loam       | 1         | 45.9                                   | 8.8              | 6.2  | 1.2           | 3.3           | 1.5           | 0.1           | 1.5†    |
|                  |            | 2         | 17.6                                   | 1.8              | 53.4   | 26.8          | 35.1          | 16.6          | 2.9           | 20.4†   |
|                  |            | 3         | 20.4                                   | 3.7              | 39.2   | 19.7          | 21.6          | 11.7          | 1.6           | 13.6†   |
|                  |            | 4         | 15.0                                   | 3.9              | 26.5   | 20.9          | 22.1          | 8.9           | 0.8           | 13.2†   |
|                  |            | 5         | 17.9                                   | 3.3              | 42.2   | 22.4          | 24.8          | 13.6          | 0.4           | 15.3†   |
|                  |            | 6         | 5.6                                    | 0.6              | **   | 24.0          | 44.6          | 20.4          | 20.2          | 27.3†   |
|                  |            | 7         | 8.9                                    | 2.5              | **   | 22.2          | 44.4          | 12.5          | 10.2          | 22.3†   |
|                  | Sandy loam | 2         | 17.7                                   | 1.5              | 52.2   | 24.4          | 43.2          | 8.4           | 2.2           | 26.1    |
|                  |            | 3         | 15.0                                   | 4.8              | 39.8   | 16.3          | 38.6          | 9.1           | 1.9           | 21.1    |
|                  |            | 4         | 17.3                                   | 4.7              | 10.0   | 17.5          | 36.1          | 9.2           | 0.5           | 14.7    |
|                  |            | 7         | 6.0                                    | 2.6              | 57.2   | 18.7          | 46.3          | 21.2          | 12.9          | 31.3    |
|                  | Clay       | 2         | 18.0                                   | 2.3              | 53.3   | 21.5          | 50.2          | 9.8           | 4.5           | 27.9    |
|                  |            | 3         | 25.7                                   | 4.8              | 55.8   | 17.6          | 43.5          | 8.1           | 1.5           | 25.3    |
|                  |            | 4         | 23.7                                   | 3.3              | 59.6   | 24.8          | 38.0          | 6.2           | 2.9           | 26.3    |
|                  |            | 7         | 8.4                                    | 2.7              | 46.4   | 42.5          | 70.4          | 47.0          | 18.4          | 44.9    |
| Red-root Pigweed | Loam       | 1         | 18.0                                   | 12.9             | 1.7  | 0.9           | 3.6           | 0.6           | 0.1           | 1.4     |
|                  |            | 2         | 14.6                                   | 4.3              | 0.9  | 38.4          | 15.9          | 11.0          | 21.2          | 17.5    |
|                  |            | 3         | 17.1                                   | 6.1              | 1.2  | 18.6          | 7.4           | 2.6           | 16.0          | 9.2     |
|                  |            | 4         | 15.9                                   | 6.6              | 1.1  | 17.4          | 10.1          | 1.0           | 14.3          | 8.8     |
|                  |            | 5         | 18.1                                   | 2.3              | 1.1  | 19.9          | 4.9           | 3.0           | 7.4           | 7.3     |
|                  |            | 6         | 8.6                                    | 2.9              | **   | 26.0          | 27.3          | 14.0          | 31.7          | 24.8    |
|                  |            | 7         | 9.7                                    | 5.1              | 3.7  | 15.3          | 14.8          | 3.6           | 14.4          | 10.4    |
|                  | Sandy loam | 2         | 22.0                                   | 4.9              | **   | 15.1          | 11.3          | 2.1           | 17.9          | 11.6    |
|                  |            | 3         | 18.0                                   | 4.9              | 1.7  | 17.9          | 8.0           | 2.7           | 12.8          | 8.6     |
|                  |            | 4         | 20.2                                   | 5.8              | **   | 11.5          | 6.2           | 2.4           | 14.2          | 8.6     |
|                  |            | 7         | 10.7                                   | 5.8              | **   | 29.6          | 38.9          | 8.4           | 18.3          | 23.8    |
|                  | Clay       | 2         | 17.0                                   | 12.1             | 22.0   | 33.4          | 10.2          | 12.6          | 29.5          | 21.5    |
|                  |            | 3         | 20.6                                   | 11.8             | 12.2   | 32.5          | 9.3           | 6.8           | 13.0          | 14.8    |
|                  |            | 4         | 14.3                                   | 12.9             | 8.2  | 22.5          | 13.1          | 6.9           | 21.5          | 14.4    |
|                  |            | 7         | 11.2                                   | 9.7              | 26.9   | 21.2          | 13.1          | 16.8          | 27.4          | 21.1    |

\* As indicated on page 355.

\*\* Samples were ruined accidentally before it was possible to complete the determination.

† That is, from Oct. 31 until the following Oct. 31, except for the 1937 to 1939 period when treatment continued for 2 years.

‡ The 1936-1937 partial results are not included in the average.

was associated with the least number of viable seeds that remained on the surface of the ground at the end of the growing season. In no case was the average number of viable seeds surviving this treatment greater than 3% of the total number of seeds that were scattered on the ground (Figure 1).

Except for Russian thistle, the deeper the seeds were buried, the substantially lower was the emergence of seedlings and correspondingly higher was the number of viable seeds that survived the burial period. Under conditions not disturbed by cultivation the average percentage of seasonal seedling emergence on loam soil was 49.3, 27.1 and 10.6 for seeds lying on the surface of the ground, buried at all depths in the upper 2.5 inch of surface soil, and buried all the way down to 6-inch depth, respec-



tively. The average percentage of seeds found viable at the end of the season was 1.2, 14.2 and 23.4 respectively. These results were relatively the same on stinkweed, wild mustard, tumbling mustard and red-root pigweed, all of which possess a relatively high state of dormancy of seeds.

The foregoing results did not apply to Russian thistle which possesses a relatively low degree of dormancy of seeds. Irrespective of the type of tillage treatment, fully 99% of the Russian thistle seeds, if in contact with the ground, germinated or lost their viability without germinating within a year. Many seeds germinated and produced seedlings if they were on or within an inch or two below the surface of the ground, but most of those buried deeper either germinated and failed to emerge or merely rotted away without germinating. Thus, the deeper the Russian thistle seeds were buried the lower was the percentage of seeds that emerged, but the percentage of viable seeds that survived one season's tillage treatment in the field varied but little. The average survival for the season was approximately 0.1% for treatments in which the seeds were not buried deeper than 2.5 inches, 0.2% for seeds initially buried at all depths down to 6-inch depth but some of which were later brought nearer to the surface by cultivation (treatment 7), and 0.5% for seeds that were initially buried at depths similar to those in treatment 7 but which were not subsequently disturbed by cultivation (treatment 6). Tillage which brought the seeds nearer to the surface had a tendency of lowering the number of dormant seeds, but the effect was so slight as to be regarded insignificant for all practical purposes.

Periodical cultivation of the soils, which, to begin with, contained weed seeds all the way down to the depth of tillage, increased the emergence of seedlings and hence decreased the number of viable seeds that remained in the soil at the end of the summer-fallow period. However, the increase in the emergence of seedlings was marked only after June 30 (Table 1). This was due to the fact that tillage brought up buried seeds to the surface after those originally on or near the surface had germinated, rather than to any other stimulating effect of cultivation on germination. The number of viable seeds remaining in the soil that had been cultivated at intervals during the growing season was substantially lower than in soil containing weed seeds at similar depths but which had not been disturbed by cultivation (Figure 1). Thus, the proportion of viable seeds that survived one season of fallow periodically cultivated to 2.5-inch depth and which contained weed seeds all the way down to that depth was on an average 5.2% for stinkweed, 3.6% for wild mustard, 13.6% for tumbling mustard, and 9.2% for red-root pigweed, as compared to 1.0, 1.1, 1.5, and 1.4% for cases where the soil was not cultivated and where weed seeds were left on the surface of the ground (Table 1). Where seeds were buried all the way down to 6-inch depth, instead of 2.5-inch depth, the proportion of viable seeds surviving fallow was substantially greater and amounted to 15.8% for stinkweed, 8.0% for wild mustard, 22.3% for tumbling mustard, and 10.4% for red-root pigweed.

Some tillage treatments were repeated on loam, sandy loam, and calcareous clay soil. The results show (Table 1) a somewhat higher emergence of seedlings on clay than on the other two soils for all types of tillage treat-



ments that were employed, but the number of viable seeds surviving the different tillage treatments was essentially the same for all soils. The reason for the higher emergence of seedlings on clay was observed to be due to the existence of a finely granulated, loose surface layer. The loam and sandy loam soils generally had a firm surface crust which tended to prevent the emergence of seedlings and caused death of some before they were able to emerge.

The percentage of seasonal seedling emergence and the percentage of viable seeds surviving the cultural treatment subtracted from 100 indicates the percentage of non-viable seeds or the percentage of seeds that were viable at the time of seeding but died in the soil without emerging. The results indicate (Table 1) that the proportion of seeds belonging collectively to the two latter classes was particularly high for all weeds included in the experiment and constituted from about 30 to 92% of the total number of seeds that were added to the soil. The mortality of seeds in the soil was found to be particularly high for Russian thistle. This was because the great majority of the seeds of this weed, when buried too deeply to emerge, died and rotted away rather than remained dormant in the soil. The non-viable seeds together with those that died in the soil during the season without germinating ranged from 56 to 92% for Russian thistle and from 30 to 75% for the other weeds. The lowest mortality was for seeds scattered on the surface of the ground and the highest for seeds buried deepest in the soil.

Packing after each tillage operation in order to reduce the drying of the surface soil usually failed to show any effect on germination or on the number of viable seeds surviving the fallow period. The same results were obtained on clay, loam, and sandy loam soils (Table 1). In one case surface packing of moist loam soil after a 1.71-inch rain caused a substantial increase in the emergence of weeds. It is evident that packing dry soil is ineffective in increasing the germination of weed seeds but packing moist soil may occasionally stimulate germination.

There was no appreciable difference in the germination nor deterioration of weed seeds in soil kept continually moist by irrigation (treatment 5) as compared with soil receiving only the natural precipitation of approximately 14.5 inches (treatment 3).

As indicated in Table 2, the earliest emergence of seedlings was from seeds scattered on the surface of the ground and the latest from seeds buried deepest in the soil. The results were essentially the same on clay, loam, and sandy loam soils. The weeds emerging earliest and in greatest numbers were Russian thistle, tumbling mustard, stinkweed, wild mustard, and red-root pigweed in the order given. The great majority of the red-root pigweed seedlings emerged, even from the surface of the ground, too late to be destroyed by tillage prior to the date of seeding of spring grain crops.

The emergence of seedlings did not occur at any one time but was spread throughout most or all of the growing season, but the peak of emergence occurred at certain fixed periods, depending on the characteristic behaviour of the weed. The greatest emergence of Russian thistle occurred

TABLE 2.—INFLUENCE OF TILLAGE TREATMENTS ON EMERGENCE OF SEEDLINGS  
(Totals for 3 soil types for years 1937-42, inclusive)

| Weed                | Treat-<br>ment<br>* | Numbers emerged during periods ending. |             |           |           |            |            |            |            |            |            |             |             |            |
|---------------------|---------------------|--|-------------|-----------|-----------|------------|------------|------------|------------|------------|------------|-------------|-------------|------------|
|                     |                     | April<br>15                            | April<br>30 | May<br>15 | May<br>30 | June<br>15 | June<br>30 | July<br>15 | July<br>31 | Aug.<br>15 | Aug.<br>31 | Sept.<br>15 | Sept.<br>30 | Oct.<br>31 |
| Russian thistle     | 1                   | 6264                                   | 2562        | 1149      | 297       | 438        | 33         | 339        | 63         | 0          | 0          | 0           | 0           | 0          |
|                     | 2                   | 595                                    | 2754        | 2343      | 250       | 159        | 91         | 87         | 79         | 1          | 0          | 0           | 0           | 0          |
|                     | 3                   | 538                                    | 2325        | 2330      | 181       | 201        | 118        | 71         | 104        | 11         | 0          | 0           | 0           | 0          |
|                     | 4                   | 675                                    | 2680        | 2627      | 256       | 172        | 158        | 84         | 81         | 11         | 0          | 0           | 0           | 0          |
|                     | 5                   | 197                                    | 1047        | 758       | 131       | 32         | 31         | 7          | 1          | 0          | 0          | 0           | 0           | 0          |
|                     | 6                   | 0                                      | 364         | 398       | 136       | 25         | 4          | 5          | 30         | 0          | 0          | 0           | 0           | 0          |
|                     | 7                   | 0                                      | 1271        | 1244      | 421       | 100        | 60         | 139        | 67         | 12         | 1          | 1           | 0           | 0          |
| Stinkweed           | 1                   | 688                                    | 3918        | 1785      | 375       | 2403       | 291        | 1059       | 690        | 204        | 33         | 6           | 126         | 15         |
|                     | 2                   | 66                                     | 2905        | 2070      | 866       | 1022       | 75         | 262        | 133        | 65         | 26         | 6           | 39          | 5          |
|                     | 3                   | 69                                     | 2909        | 1704      | 719       | 1146       | 299        | 580        | 479        | 438        | 157        | 64          | 357         | 18         |
|                     | 4                   | 50                                     | 3230        | 1901      | 628       | 1439       | 315        | 526        | 565        | 388        | 73         | 77          | 331         | 30         |
|                     | 5                   | 66                                     | 3918        | 2010      | 1509      | 615        | 333        | 228        | 441        | 303        | 57         | 141         | 327         | 36         |
|                     | 6                   | 0                                      | 450         | 837       | 252       | 429        | 72         | 171        | 33         | 33         | 12         | 0           | 6           | 0          |
|                     | 7                   | 0                                      | 886         | 1324      | 363       | 535        | 628        | 772        | 416        | 373        | 102        | 37          | 219         | 8          |
| Wild mustard        | 1                   | 12                                     | 3687        | 2082      | 138       | 1773       | 480        | 1779       | 192        | 69         | 15         | 6           | 3           | 0          |
|                     | 2                   | 40                                     | 3423        | 2436      | 265       | 637        | 73         | 468        | 25         | 6          | 14         | 2           | 2           | 1          |
|                     | 3                   | 20                                     | 3675        | 2316      | 190       | 668        | 298        | 824        | 122        | 117        | 34         | 12          | 61          | 10         |
|                     | 4                   | 14                                     | 3546        | 2501      | 214       | 677        | 206        | 737        | 189        | 70         | 13         | 2           | 32          | 3          |
|                     | 5                   | 0                                      | 4218        | 2604      | 714       | 609        | 465        | 213        | 231        | 66         | 18         | 21          | 18          | 3          |
|                     | 6                   | 0                                      | 783         | 1362      | 255       | 630        | 30         | 201        | 12         | 18         | 9          | 3           | 6           | 0          |
|                     | 7                   | 0                                      | 1139        | 2668      | 333       | 293        | 408        | 809        | 195        | 67         | 13         | 1           | 48          | 4          |
| Tumbling<br>Mustard | 1                   | 1986                                   | 6849        | 1443      | 93        | 282        | 18         | 267        | 555        | 6          | 12         | 39          | 90          | 1554       |
|                     | 2                   | 107                                    | 2614        | 1195      | 121       | 110        | 26         | 95         | 115        | 0          | 1          | 29          | 76          | 61         |
|                     | 3                   | 191                                    | 3672        | 905       | 119       | 50         | 26         | 32         | 111        | 28         | 63         | 56          | 262         | 456        |
|                     | 4                   | 124                                    | 2966        | 847       | 160       | 87         | 36         | 27         | 102        | 56         | 47         | 60          | 186         | 423        |
|                     | 5                   | 78                                     | 3075        | 837       | 396       | 180        | 15         | 6          | 3          | 15         | 78         | 72          | 234         | 321        |
|                     | 6                   | 0                                      | 435         | 846       | 18        | 27         | 3          | 63         | 84         | 0          | 12         | 3           | 6           | 3          |
|                     | 7                   | 0                                      | 775         | 935       | 52        | 29         | 57         | 30         | 17         | 43         | 95         | 35          | 136         | 229        |
| Red-root<br>Pigweed | 1                   | 0                                      | 108         | 732       | 465       | 1626       | 675        | 2421       | 1188       | 6          | 81         | 27          | 3           | 3          |
|                     | 2                   | 0                                      | 0           | 593       | 1176      | 1363       | 636        | 1285       | 623        | 70         | 49         | 9           | 11          | 0          |
|                     | 3                   | 0                                      | 152         | 685       | 771       | 1700       | 1223       | 926        | 646        | 183        | 150        | 27          | 34          | 0          |
|                     | 4                   | 0                                      | 0           | 398       | 1164      | 1428       | 938        | 1246       | 663        | 181        | 68         | 23          | 51          | 4          |
|                     | 5                   | 0                                      | 0           | 720       | 1482      | 1068       | 756        | 141        | 195        | 60         | 42         | 84          | 21          | 0          |
|                     | 6                   | 0                                      | 0           | 216       | 402       | 966        | 144        | 600        | 111        | 6          | 105        | 0           | 0           | 0          |
|                     | 7                   | 0                                      | 00          | 447       | 457       | 692        | 746        | 1151       | 457        | 80         | 40         | 6           | 50          | 0          |

\* As indicated on page 355.

† No weeds emerged between Oct. 31 and March 31.

early in the spring, except where seeds were buried deeply in the soil, in which case emergence was delayed considerably. Hardly any germination occurred after August 15.

Substantial numbers of seedlings of other weeds, except red-root pigweed, likewise emerged in large numbers in the spring, though emergence of many seedlings continued throughout the whole of the growing season. The second peak of emergence, though not as pronounced as the first, occurred in the fall for stinkweed and tumbling mustard, both of which behave as winter annuals to some degree.



The distribution of seasonal emergence of red-root pigweed was entirely different from that of other weeds included in the experiment. The seeds did not germinate in appreciable numbers until late spring and summer. This is why the weed is particularly prevalent on land that is being fallowed, for it emerges in large numbers after many other weeds have more or less ceased to germinate, thus necessitating additional tillage to keep it down.

### DISCUSSION AND CONCLUSIONS

The reduction in the number of viable seeds by one season of summer-fallow varied inversely with the depth of burial of weed seeds, the highest reduction being from seeds lying on the surface of the ground. These results indicate very forcefully (Figure 1) that tillage which tends to bury the seeds, even at shallow depths, has a definite effect of lowering the germination of seeds and of *inducing* a substantial proportion of the seeds to remain dormant in the soil for periods longer than if they were left on the surface of the ground. Perpetuation of many small seeded weeds is thus accentuated by cultivation.

The foregoing results and conclusions apply only to small weed seeds possessing a relatively high degree of dormancy, but do not apply to seeds of relatively low degree of dormancy, such as those of Russian thistle, nor possibly for relatively large seeds such as those of wild oats. The type of tillage employed appears to be of no consequence in reducing the infestations of Russian thistle seeds, because the great majority of those lying on or within an inch or two below the surface of the ground germinate as soon as conditions become suitable, but those buried deeper either germinate and fail to emerge or merely rot away without germination. The results indicate that, on an average, substantially less than 1% of Russian thistle seeds, if in contact with the ground remain viable at the end of the fallow period, irrespective of what depth the fallow is worked. Because of the relatively low dormancy of the seeds, infestations can be readily eliminated, provided no plants are allowed to produce seeds and mature plants are prevented from being carried into the treated area by the wind.

The results show that for weeds exhibiting a relatively high degree of dormancy of seeds, the number of viable seeds surviving one season of fallow that has been cultivated at intervals during the growing season is substantially lower than in soil containing the weed seeds at similar depths but which has not been disturbed by cultivation. Repeated cultivation thus partially offsets the unfavourable effects of the initial cultivation that has buried some or all of the seeds. As indicated by experimental results, however, the number of viable seeds remaining at the end of the season in cultivated soil is substantially higher than in the case where the soil is not cultivated at all and in which all weed seeds remain on the surface of the ground. This suggests that shallow tillage, such as with a suitable type of blade implement that has the least tendency of burying the seeds, should allow for greater emergence of small seeded weeds than tillage which tends to stir the soil and thus bury the seeds. In any case, tillage should be as shallow as possible, consistent with effective destruction of growing weeds.



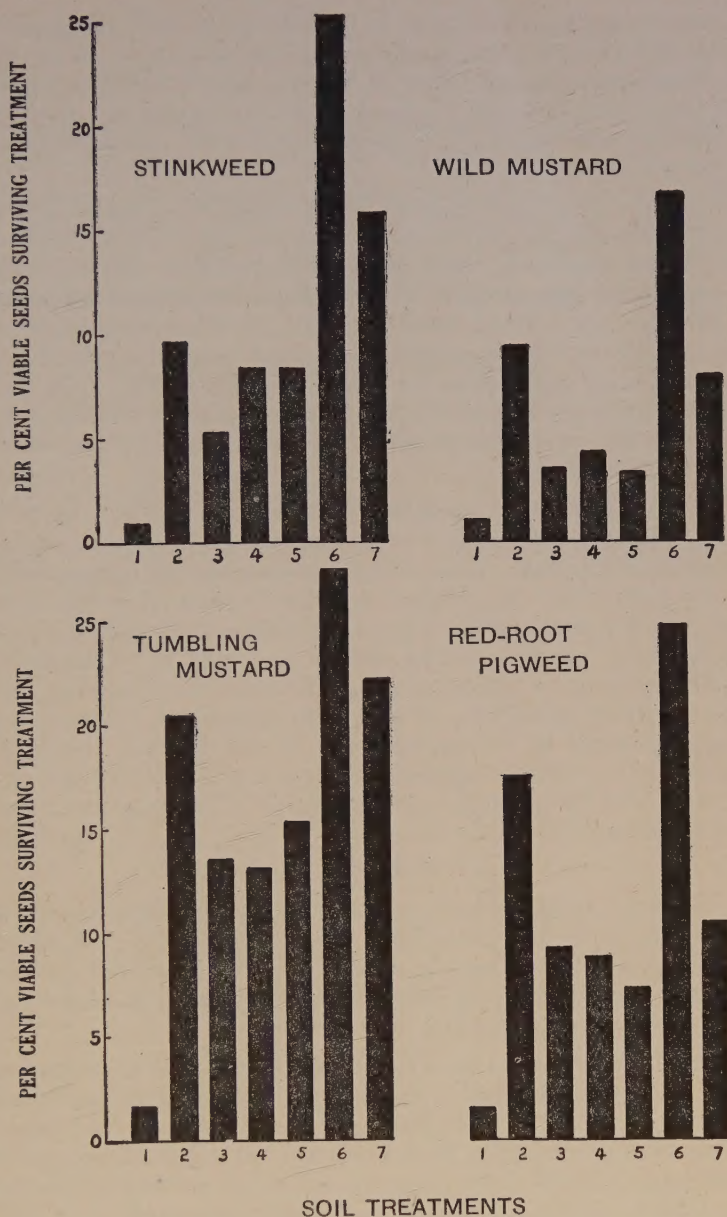


FIGURE 1.—Average percentage of viable seeds surviving one season of tillage treatment on loam soil, as follows: 1. Seeds scattered on the surface, no tillage. 2. Seeds mixed in 2.5-inch layer of soil, no tillage. 3. Seeds mixed in 2.5-inch layer of soil, cultivated 4 times to 2.5-inch depth. 4. Seeds mixed in 2.5-inch layer of soil, cultivated 4 times to 2.5-inch depth and packed. 5. Seeds mixed in 2.5-inch layer of soil, cultivated 4 times to 2.5-inch depth and watered to keep continually moist. 6. Seeds mixed in 6-inch layer of soil, no tillage. 7. Seeds mixed in 6-inch layer of soil, ploughed June 1, cultivated 3 times to 2.5-inch depth.

The results indicate that packing of fallow after each tillage operation seldom has any effect on the germination of weed seeds or on the number of viable seeds surviving the fallow period. A beneficial effect is occasionally achieved on moist soil but the results are too irregular and of insufficient benefit to warrant the adoption of this method into the regular farm practice on dryland soils.

Contrary to what was expected, there appeared to be no appreciable effect of irrigation on the germination or on the longevity of weed seeds in the soil. The longevity of weed seeds on periodically cultivated dryland soils is apparently due to their natural and induced dormancy and not to the limited supply of soil moisture. At one time or another there is sufficient rainfall during the growing season to cause the germination, or disintegration, of all seeds whose dormancy has been broken. On the other hand, an increase in precipitation over and above that required to germinate the weed seeds does not seem to affect the natural nor induced dormancy of seeds.

The results show that the earliest emergence of seedlings in the spring is from seeds lying on the surface of the ground and the latest from seeds buried deepest in the soil. This brings out another argument for shallow tillage. In order that the greatest possible number of young weeds be destroyed before a spring crop is sown, it is necessary, therefore, to have small seeds on or as near to the surface as possible. The practice of delaying the seeding of spring crops until many weed seedlings can be destroyed by tillage should be of particular benefit in combating weeds that germinate early in the spring, such as the Russian thistle. Some weeds, such as the red-root pigweed, emerge too late in the spring, even from the surface of the ground, to be destroyed by tillage prior to the most suitable date of seeding.

#### SUMMARY

The influence of tillage treatments on the longevity of weed seeds in the soil was found to depend in large measure on the period of induced dormancy of seeds. Weeds may be divided into two broad categories, those possessing a relatively short period of induced dormancy of seeds and those possessing a relatively long period.

For small seeds possessing a relatively long period of dormancy, the deeper the seeds were buried in the soil the substantially lower was the emergence of seedlings and correspondingly higher was the number of viable seeds that survived the burial period. The highest emergence and the lowest percentage of viable seeds remaining at the end of the growing season was from seeds lying on the surface of the ground.

For seeds possessing a relatively short period of dormancy, such as those of Russian thistle, the depth of burial was of little consequence, for those buried too deeply to emerge soon lost their viability in any case.

Periodical cultivation that brought weed seeds nearer to the surface after those originally on or near the surface have germinated, increased the emergence of seedlings and decreased the number of viable seeds in the soil. The treatment was not as effective, however, as if all weed seeds were left on the surface of undisturbed soil.



There was higher emergence of seedlings from calcareous clay soil than from loam or sandy loam, but the number of viable seeds surviving different cultural treatments was essentially the same in all soils.

Packing after each tillage operation to stimulate the germination of weed seeds was usually ineffective on dryland soils.

Irrigation, in addition to natural precipitation, had no appreciable effect on germination nor on longevity of weed seeds in the soil.

The earliest emergence of seedlings took place from seeds lying on the surface of the ground and the latest from seeds buried deepest in the soil.

#### ACKNOWLEDGMENT

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#### REFERENCE

1. CHEPIL, W. S. Germination of weed seeds. I. Longevity, periodicity of germination, and vitality of seeds in cultivated soil. *Sci. Agr.* 26 : 307-346. 1946.

# ACTIVITY OF PATULIN AGAINST *USTILAGO TRITICI* (PERS.) JEN.<sup>1</sup>

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Continuing the search for new antibiotics of microbial origin, one species of *Penicillium* isolated from mummified round-headed wood borer (*Cerambycidae*) when grown on modified Czapek-Dox liquid medium, produced a metabolism solution active against *Staphylococcus aureus* and *Escherichia coli*.

The metabolism solution produced by this mould was sent to Dr. W. D. McFarlane who isolated the active matter in crystalline form and identified it as patulin.

The name of this chemical compound "patulin" was given by Raistrick *et al.* (16) who isolated this antibiotic from a metabolism solution of *Penicillium patulum* Bainier, and who also elucidated the chemical nature of the patulin as anhydro-3-hydroxymethylene-tetrahydro-1 : 4-pyrone-2-carboxylic acid. Later it was shown by Bergel *et al.* and others (2, 5, 11, 13) that patulin was identical with the antibiotic produced by *Aspergillus clavatus* and previously designated as clavacin by Waksman *et al.* (24) and as clavatin by Bergel *et al.* (3), and also that formed by *Penicillium claviforme* to which Chain *et al.* (4) had already given the name claviformin<sup>3</sup>. According to recent reports it seems that patulin is a common metabolic by-product of many organisms. Thus Florey *et al.* (7) obtained it from *Aspergillus giganteus*, Karrow and Foster (12) from *Gymnoascus*, Kent and Heatley (14) from *Penicillium utricae* and *Aspergillus terreus*, Lochhead *et al.* (15) from soil *Penicillia*, Timonin and McFarlane (22) from *Byssoclamys* sp. and Anslow *et al.* (1) from *Penicillium expansum*.

The activity *in vitro* of patulin, claviformin, clavacin and clavatin against Gram positive and Gram negative bacteria has been reported by the above mentioned authors. Furthermore, several workers (1, 9, 17, 23) also reported on the antifungal activity of patulin *in vitro*. In this laboratory, when patulin was made available in crystalline form and its activity against bacteria and certain fungi was established, the investigation on the activity of patulin against loose smut of wheat was outlined with the object of obtaining information on the possibility of practical control of loose smut of wheat with the aid of patulin.

## EXPERIMENTAL

The *Penicillium* sp. when grown on modified Czapek-Dox (glucose 40 gm.;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  0.25 gm.;  $\text{NaNO}_3$  3 gm.;  $\text{KH}_2\text{PO}_4$  0.5 gm.;  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$  0.006 gm.;  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  0.006 gm.) Raulin-Thom and peptone glucose (peptone 10 gm.; commercial glucose "grape sugar" 40 gm.) liquid media in stationary or submerged cultures produced a metabolism solution as indicated in Table 1 with considerable activity. The assay for

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<sup>3</sup> There is at present lack of agreement as to the correct designation of this antibiotic. In this paper the term "patulin" is used since that was the name under which the crystalline material was furnished by Dr. McFarlane.



TABLE 1.—INFLUENCE OF MEDIUM ON PRODUCTION OF ANTIBIOTIC

| Medium   | Culture   | Zone of inhibition,<br>mm./diam. |                |
|--|-----------|----------------------------------|----------------|
|  |           | <i>Staph.<br/>aureus</i>         | <i>E. coli</i> |
| Czapek-Dox + glucose (40 gm./l.) Med I             | Surface   | 35                               | 32             |
|  | Submerged | 30                               | 30             |
| Czapek-Dox + honey (50 gm./l.) Med. II             | Surface   | 40                               | 40             |
|  | Submerged | 37                               | 38             |
| Czapek-Dox + comm. glucose (40 gm./l.)<br>Med. III | Surface   | 32                               | 30             |
|  | Submerged | 28                               | 29             |
| Med. I + peptone (10 gm./l.)                       | Surface   | 25                               | 26             |
|  | Submerged | 0                                | 0              |
| Med. II + peptone (10 gm./l.)                      | Surface   | 28                               | 29             |
|  | Submerged | 0                                | 0              |
| Med. III + peptone (10 gm./l.)                     | Surface   | 30                               | 29             |
|  | Submerged | 0                                | 0              |
| Med. I + potato extract (25 ml./l.)                | Surface   | 40                               | 39             |
|  | Submerged | 37                               | 36             |
| Med. II + potato extract (25 ml./l.)               | Surface   | 41                               | 42             |
|  | Submerged | 38                               | 40             |
| Med. III + potato extract (25 ml./l.)              | Surface   | 36                               | 37             |
|  | Submerged | 32                               | 34             |
| Raulin-Thom  | Surface   | 31                               | 30             |
|  | Submerged | 32                               | 30             |
| Peptone 10 gm.; commer. glucose (40 gm./l.)        | Surface   | 28                               | 29             |
|  | Submerged | 0                                | 0              |

potency of these metabolism solutions was carried out by the standard cup method as outlined by Schmidt and Moyer (19). The maximum activity of metabolism solutions in stationary cultures was obtained on the 8th to 10th day of incubation whereas in submerged cultures it developed on the 6th and 7th day of incubation at 27–28° C.

From the data presented in Table 1 it is evident that addition of peptone (10 gm./l.) to the media in surface cultures caused the reduction in potency of the metabolism solution and complete inhibition of production of antibiotic in submerged cultures. On the other hand addition of 25 ml./l. of potato extract to the modified Czapek-Dox medium resulted not only in the increase in potency of the metabolism solution but also enhanced the germination of the spores and growth of mycelium. Thus the cultures with potato extract produced surface mycelial pellicle in less than 24 hours, whereas cultures without potato extract required 48 to 56 hours' incubation to produce the same type of pellicle. The best yield of crystalline patulin, 1.325 gm./l., was obtained from the cultures containing potato extract. The potato extract was prepared according to the method outlined by Robbins *et al.* (18).

ACTIVITY OF CRYSTALLINE PATULIN

Patulin produced by this organism was active against Gram positive and Gram negative bacteria. Thus it inhibited the growth of *Staph. aureus* H. in 1 : 125,000 and *Escherichia coli* in 1 : 160,000 dilution. However, the addition of 5% fresh horse serum reduced this activity by about  $\frac{2}{3}$  of the original, and the activity was completely destroyed when serial dilutions, prepared in nutrient broth, were autoclaved for 15 min. at 15 lb. pressure, (21, 22).

The activity of patulin has been also assayed against several fungi. The data presented in Table 2 indicate that patulin in 1 : 20,000 dilution, reduced the rate of growth of several fungi, namely, *Ascochyta pinodes*,

TABLE 2.—FUNGISTATIC ACTIVITY OF PATULIN *in vitro*

|                                  | Dilution of patulin (1000) |      |      |      |      |      |         |
|----------------------------------|----------------------------|------|------|------|------|------|---------|
|                                  | 20                         | 40   | 50   | 66   | 80   | 100  | Control |
| <i>Ascochyta pinodes</i>         | ++                         | ++   | +++  | ++++ | ++++ | ++++ | +++     |
| <i>Ascochyta pinodetta</i>       | ++++                       | ++++ | ++++ | ++++ | ++++ | ++++ | ++++    |
| <i>Ascochyta pisi</i>            | ++                         | ++   | ++   | ++++ | ++++ | ++++ | ++++    |
| <i>Fusarium culmorum</i>         | ++++                       | ++++ | ++++ | ++++ | ++++ | ++++ | ++++    |
| <i>Helminthosporium sativum</i>  | ++                         | ++++ | ++++ | ++++ | ++++ | ++++ | ++++    |
| <i>Rhizoctonia solani</i>        | ++++                       | ++++ | ++++ | ++++ | ++++ | ++++ | ++++    |
| <i>Ustilago Tritic</i>           | —                          | —    | —    | —    | —    | —    | ++++    |
| <i>Blastomyces Gilchristi</i>    | +++                        | +++  | +++  | +++  | +++  | +++  | ++++    |
| <i>Epidermophyton floccosum</i>  | +                          | ++   | ++   | +++  | ++++ | ++++ | ++++    |
| <i>Microsporum lanosum</i>       | ++                         | ++   | +++  | ++++ | ++++ | ++++ | ++++    |
| <i>Trichophyton crateriforme</i> | ++                         | +++  | +++  | +++  | ++++ | ++++ | ++++    |
| <i>Trichophyton gypsum</i>       | ++                         | +++  | ++++ | ++++ | ++++ | ++++ | ++++    |
| <i>Trichophyton purpureum</i>    | +++                        | +++  | ++++ | ++++ | ++++ | ++++ | ++++    |

- No growth.
- + Very poor growth.
- ++ Poor growth.
- +++ Good growth.
- ++++ Normal growth.

*A. pisi*, *Epidermophyton floccosum*, *Microsporum lanosum*, *Trichophyton crateriforme*, *T. gypsum*, and completely inhibited the growth of *Ustilago Tritic*.

Originally the spores (chlamydospores\*) of *Ustilago Tritic* were received from the Dominion Rust Research Laboratory, Winnipeg, Manitoba, and were marked as "Strain I". This strain was used by the Cereal Division, Central Experimental Farm, Ottawa, for a study of the resistance of wheat hybrids to loose smut and the material (smutted plants) used in this work was obtained from the Cereal Division.

ACTIVITY OF PATULIN AGAINST *Ustilago tritici*

The activity of patulin was assayed against this fungus in mycelial stage, by the dilution method and the following results were obtained:

- Fungistatic — 1 : 400,000
- Fungicidal — 1 : 100,000

\* The chlamydospores of *Ustilago Tritic* in this paper will be referred to as spores.



The fungicidal activity was determined by transferring the original bit of inoculum after 5 to 10 days' incubation on fresh patulin-free medium and the limiting dilution for complete inhibition of growth was recorded as fungicidal activity.

The activity of patulin was also determined against the spore stage. Results summarized in Table 3 indicate that complete inhibition of germination of spores was obtained in 1 : 66,000 dilution and partial inhibition (56% spores germinated) in 1 : 200,000 dilution. These results were obtained by the following method: spores of the fungus, obtained from the smutted wheat heads, were suspended in the serial patulin dilution and 1 ml. of suspension of each serial dilution was poured on the surface of potato dextrose (2%) agar in Petri dishes. By rotating the plates the spores were distributed over the agar surface. The plates were then incubated at 20° C. for 24 and 48 hours and with the aid of a microscope the germinated and dead spores were counted in each microscope field of vision. Thus the spores in 25 microscope fields were counted and the percentage of germinated spores for each dilution calculated. The results are summarized in Table 3.

TABLE 3.—ACTIVITY OF PATULIN AGAINST SPORES OF  
*Ustilago Triticis*

| Concentration of<br>patulin | Germinated spores |                |
|-----------------------------|-------------------|----------------|
|                             | 24 hrs. incub.    | 48 hrs. incub. |
|                             | %                 | %              |
| 1 : 4,000                   | 0                 | 0              |
| 1 : 8,000                   | 0                 | 0              |
| 1 : 10,000                  | 0                 | 0              |
| 1 : 20,000                  | 0                 | 0              |
| 1 : 40,000                  | 0                 | 0              |
| 1 : 50,000                  | 0                 | 0              |
| 1 : 66,000                  | 0                 | 0              |
| 1 : 80,000                  | 0                 | 7.7            |
| 1 : 100,000                 | 7.6               | 11.2           |
| 1 : 133,000                 | 28.5              | 29.5           |
| 1 : 200,000                 | 45.4              | 55.8           |
| Control                     | 51.9              | 85.8           |

During examination of the plates it was noticed that plates poured with spore suspensions containing patulin in 1 : 100,000 dilution and lower were free from bacterial contamination, whereas control plates and plates with patulin in dilutions higher than 1 : 100,000 were badly contaminated. After 48 hours' incubation the counting of spores on control plates was very difficult. Thus the use of patulin in isolation of loose smut of wheat in pure culture may be recommended.

The effect of pretreatment in patulin solution on the viability of the spores was also investigated. In order to obtain information, in this case, the spores were suspended in serial patulin dilutions and allowed to stand at room temperature for 2, 4, 8, 12 and 24 hours. The samples were then centrifuged, supernatant liquid decanted, spores resuspended in sterile

tap water and suspensions were again centrifuged. To insure complete removal of patulin from the spores this process was repeated twice. One ml. of patulin-free spore suspension was then poured on the surface of solidified agar in Petri dishes and after 24 and 48 hours' incubation the germinated and dead spores, with the aid of the microscope, were counted. Control samples were passed through the same procedure only in sterile tap water.

Results summarized in Table 4 indicate that the percentage of germinated spores is in reverse correlation with the concentration of patulin and length of time of pretreatment. The highest dilution which completely inhibited the germination of spores after 24 hours' pretreatment was 1 : 20,000.

TABLE 4.—GERMINATION OF SPORES AS INFLUENCED BY TIME OF PRETREATMENT AND CONCENTRATION OF PATULIN SOLUTION

| Concentration of patulin solution | Counted after 24 hrs. incub.    |       |       |       |       | Counted after 48 hrs. incub. |       |       |       |       |
|-----------------------------------|---------------------------------|-------|-------|-------|-------|------------------------------|-------|-------|-------|-------|
|                                   | Time of treatment—hours         |       |       |       |       | Time of treatment—hours      |       |       |       |       |
|                                   | 2                               | 4     | 8     | 12    | 24    | 2                            | 4     | 8     | 12    | 24    |
|                                   | Percentage of germinated spores |       |       |       |       |                              |       |       |       |       |
| 1 : 4,000                         | 0                               | 0     | 0     | 0     | 0     | 0                            | 0     | 0     | 0     | 0     |
| 1 : 8,000                         | 10.50                           | 8.50  | 0.10  | 0.04  | 0.0   | 18.35                        | 10.13 | 2.45  | 0.06  | 0.0   |
| 1 : 20,000                        | 22.00                           | 15.35 | 0.78  | 0.38  | 0.0   | 24.75                        | 10.23 | 1.02  | 0.65  | 0.0   |
| 1 : 40,000                        | 35.18                           | 18.12 | 1.56  | 0.98  | 0.93  | 38.39                        | 23.45 | 4.36  | 3.53  | 2.73  |
| 1 : 50,000                        | 47.35                           | 31.34 | 6.58  | 2.93  | 1.34  | 56.45                        | 38.03 | 9.53  | 7.35  | 2.86  |
| 1 : 66,000                        | 69.12                           | 38.43 | 29.38 | 19.75 | 15.43 | 72.76                        | 45.45 | 31.32 | 31.42 | 20.40 |
| 1 : 80,000                        | 72.45                           | 48.24 | 37.13 | 21.15 | 19.01 | 70.31                        | 51.34 | 46.51 | 28.32 | 24.80 |
| 1 : 100,000                       | 73.34                           | 65.85 | 43.75 | 30.45 | 23.25 | 74.42                        | 68.34 | 56.34 | 36.15 | 27.93 |
| Control (water)                   | 72.84                           | 74.35 | 75.26 | 72.83 | 73.46 | 75.36                        | 72.86 | 86.43 | 84.15 | 84.44 |

#### EFFECT OF PATULIN ON GERMINATION OF WHEAT SEED

In view of the possibility of utilization of patulin in practical control of loose smut of wheat, the effect of patulin on the germination of wheat seed was investigated. Wheat seeds, healthy in appearance and not

TABLE 5.—EFFECT OF PATULIN ON GERMINATION OF WHEAT SEED

| Concentration of patulin solution | Planted while wet |      |       | Planted after drying |      |       |
|-----------------------------------|-------------------|------|-------|----------------------|------|-------|
|                                   | Germination       |      |       | Germination          |      |       |
|                                   | Strong            | Weak | Total | Strong               | Weak | Total |
|                                   | %                 | %    | %     | %                    | %    | %     |
| 1 : 4,000                         | 42                | 4    | 46    | 37                   | 8    | 45    |
| 1 : 8,000                         | 77                | 7    | 84    | 71                   | 10   | 81    |
| 1 : 20,000                        | 90                | 5    | 95    | 86                   | 7    | 93    |
| Water (control)                   | 93                | 5    | 98    | 91                   | 6    | 97    |
| Control untreated                 |                   |      |       | 93                   | 6    | 99    |
| Control untreated                 |                   |      |       | 93                   | 4    | 97    |



TABLE 6.—INFLUENCE OF TREATMENT ON THE WEIGHT OF WHEAT SEED

| Time soaking,<br>hours | Tap water          |                    |          | Patulin—1 : 20,000 solution |                    |          |
|------------------------|--------------------|--------------------|----------|-----------------------------|--------------------|----------|
|                        | Wt. of<br>dry seed | Wt. of<br>wet seed | Increase | Wt. of<br>dry seed          | Wt. of<br>wet seed | Increase |
|                        | gm.                | gm.                | %        | gm.                         | gm.                | %        |
| 2                      | 6.90               | 7.90               | 14.49    | 6.95                        | 7.95               | 14.39    |
| 4                      | 6.80               | 8.16               | 20.00    | 6.85                        | 8.10               | 18.25    |
| 8                      | 7.00               | 8.80               | 25.71    | 7.00                        | 8.75               | 25.00    |
| 12                     | 7.10               | 9.30               | 30.99    | 7.05                        | 9.20               | 30.50    |
| 24                     | 6.96               | 10.16              | 45.98    | 6.90                        | 9.70               | 40.58    |
| Average of total       | 6.95               | 8.86               | 28.03    | 6.95                        | 8.74               | 25.74    |

TABLE 7.—GERMINATION OF WHEAT SEED AS INFLUENCED BY LENGTH OF TIME OF TREATMENT

| Treatment<br>in hours | Water       |             | Patulin—1 : 20,000 solution |             |
|-----------------------|-------------|-------------|-----------------------------|-------------|
|                       | Germination |             | Germination                 |             |
|                       | Planted dry | Planted wet | Planted dry                 | Planted wet |
|                       | %           | %           | %                           | %           |
| 2                     | 96          | 97          | 95                          | 98          |
| 4                     | 100         | 99          | 98                          | 99          |
| 8                     | 96          | 99          | 95                          | 96          |
| 12                    | 96          | 96          | 94                          | 95          |
| 24                    | 98          | 98          | 93                          | 94          |
| Total                 | 486         | 489         | 472                         | 483         |
| Average               | 97.20       | 97.80       | 94.40                       | 96.60       |
| Control untreated     | 98          |             | 97                          |             |
| Average               | 97.5        |             |                             |             |

injured by threshing, were subjected to the following treatment. Four samples of seed, 200 seeds each, were soaked for 24 hours in 1 : 4,000, 1 : 8,000, 1 : 20,000 and in tap water respectively. After treatment each sample was divided into 2 lots (100 seeds each) and one lot was sown in flats in greenhouse soil immediately after soaking, while the second lot prior to sowing was allowed to dry at room temperature to the original weight.

From the results obtained (Table 5) it is evident that patulin in concentrations used is toxic to the wheat embryo. Thus 1 : 4,000 dilution reduced germination by 53.60 and 53.06%, 1 : 8,000 by 16.50 and 14.29% and 1 : 20,000 by 4.12 and 3.98% of dry and wet planting respectively, when the germination of control samples (water soak) is expressed as 100%.

From results obtained it is evident that patulin in 1 : 20,000 dilution only slightly reduces the germination of wheat seed and this dilution was used in further experiments.

To study the effect of length of time of soaking on the germination of wheat seed the samples of wheat, 200 seeds in each treatment, were soaked in 1 : 20,000 dilution for 2, 4, 8, 12 and 24 hours. Control samples were kept in tap water for a corresponding length of time. In order to obtain rates of adsorption of patulin solution and water for different periods of time of soaking the samples were weighed before and after treatment. After treatment and before weighing the surface of the seeds was dried on blotting paper. The results of this experiment summarized in Tables 6 and 7 indicate that the rate of absorption of patulin solution was lower than absorption of the water. The difference, after 24 hours soaking amounted to 5.4%. The percentage of germinated seeds in patulin treated lots was also lower, amounting after 24 hours soaking to 5.71 and 4.08% of dry and wet seed planting respectively.

#### EFFECT OF PATULIN ON LOOSE SMUT *in situ*

In order to obtain some information on the effect of patulin on the loose smut *in situ*, artificially infected wheat seeds, obtained from Cereal Division, Ottawa, were soaked in patulin solution (1 : 20,000) for 24 hours and controls soaked in tap water for the same length of time. After treatment the samples were again divided into 2 lots and 1 lot was sown immediately after treatment in greenhouse soil, in 7-inch pots, 10 seeds to the pot, while the second lot prior to sowing was dried to the original weight. Control without treatment was also included.

Plants were grown in the greenhouse and watered with tap water. The emergence of seedlings for each treatment was recorded and later, at heading time, the smutted plants were counted and the percentage of infection calculated. Results of this experiment are summarized in Table 7. From the data presented it is apparent that the emergence in patulin treated samples was 4.09 and 3.06% lower than in control treatment of dry and wet seed planting respectively. This reduction again illustrates the phytocidal effect of patulin on the wheat embryo. Furthermore, the results also clearly indicate that patulin 1 : 20,000 dilution does not control loose smut *in situ*.

This experiment was repeated with different seed material and a new set of data was obtained but the trend of infection remained the same.

It is of interest to note that the fungicidal activity of patulin *in vitro* against mycelium and spores as determined is 1 : 100,000 and 1 : 20,000 (1 : 60,000) respectively and the reduction in germination of patulin treated seeds indicate that patulin solution was able to diffuse through the seed coat. In spite of these facts the pathogen *in situ* was not controlled by patulin. In explanation of this, two possibilities could be suggested (1) that patulin was inactivated by the organo-chemical complex of the wheat kernel, and (2) that the resting stage of mycelium imbedded in the scutellum is resistant to patulin.

According to Cavallito *et al.* (6) and Geiger and Conn (8) chemical compounds containing the sulfhydryl group inactivate patulin *in vitro*. In this respect grain does contain a considerable amount of proteins the chemical formulae of which contain the sulfhydryl group. To investigate the possibility that patulin was inactivated by the content of the wheat



kernel, whole flour was prepared from the parent grain of the hybrid seed and different amounts of it were added to 50 ml. of 1 : 20,000 patulin solution. The suspensions after 24 hours' standing at room temperature were filtered through filter paper, the soluble proteins coagulated by heat (10 min. in boiling water bath) and filtered off. The activity of patulin was then assayed against *Staph. aureus*. It was found (Table 9) that an addition of 10 gm. of flour to 50 ml. of patulin solution reduced the activity by  $\frac{4}{5}$  of the control, which was passed through the same procedure, except for the addition of flour. The results, therefore, indicate that part of the patulin was inactivated by the content of the wheat kernel. Plant pathologists, on the other hand, have demonstrated (20) that the hyphae of the pathogen in the majority of cases are imbedded in the scutellum of the wheat kernel, and if the chemical composition of the scutellum and the embryo are considered a third explanation may be suggested.

According to Hinton (10) the wheat kernel on an average, contains 1.5%, by weight, of scutellum and 1.2% of embryo. Furthermore he stated that number one Manitoba contained 9.3 and 168  $\gamma$ /gm. vitamin B<sub>1</sub> and 15.4 and 30.3% fat lipoids in embryo and scutellum respectively. Taking his data as a basis for calculation, 200 wheat kernels weighing 6.9 gm. would contain 0.77 and 17.39 $\gamma$  of vitamin B<sub>1</sub> and 0.0128 and 0.0314 gm. of fat lipoids in embryo and scutellum respectively. The addition of 1.9 $\gamma$ /ml. of vitamin B<sub>1</sub> to the assay medium resulted in complete inhibition of the activity of patulin and furthermore, it was found that patulin is not

TABLE 8.—EFFECT OF PATULIN ON *Ustilago tritici* *in situ*

| Treatment              | Emergence | Total plants harvested | Smutted plants | Infected |
|------------------------|-----------|------------------------|----------------|----------|
|                        | %         |                        |                | %        |
| Control (untreated)    | 96        | 93                     | 23             | 24.73    |
| Water (planted wet)    | 98        | 95                     | 19             | 20.00    |
| Water (planted dry)    | 98        | 91                     | 19             | 20.08    |
| Patulin* (planted wet) | 95        | 92                     | 21             | 22.83    |
| Patulin* (planted dry) | 94        | 91                     | 23             | 25.27    |

\* Patulin = 1 : 20,000 solution.

TABLE 9.—ACTIVITY OF PATULIN AGAINST *Staph. aureus*  
AS INFLUENCED BY WHEAT FLOUR

| Weights of flour added per 50 ml. (1 : 20,000) patulin solution | Limiting dilutions showing complete inhibition of growth |
|---|--|
| gm.   |  |
| 10.0  | 1 : 25,000   |
| 5.0   | 1 : 66,000 $\pm$   |
| 2.5   | 1 : 66,000   |
| 1.0   | 1 : 100,000  |
| 0.0 (control)   | 1 : 125,000  |

soluble in wheat germ oil. Therefore it may be suggested that the scutellum due to the high content of vitamin B<sub>1</sub> and fat lipoids, protects the imbedded mycelium from the activity of the patulin.

On the other hand the chemical composition and degree of resistance of the mycelium imbedded in scutellum to patulin is not known at present. However, taking the results presented in Table 8 into consideration and comparing them with results published by Zalkssky (25) the reduction in percentage of infection due to the water treatment suggests that the mycelium some time during the 24 hours' soaking passed from the dormant stage into the active one, and owing to lack of oxygen a certain percentage of mycelium died as shown in this paper (reduction in percentage of infection), and was completely eliminated under anaerobic conditions as in Zalkssky's experiments. If, therefore, during the 24-hour treatment the mycelium was in the active stage additional support is given to the theory of protection of mycelium by scutellum; because the active mycelium *in vitro* is very susceptible to patulin.

### SUMMARY

The effect of the composition of the medium on yield of patulin by *Penicillium* sp. in surface and submerged cultures has been described and the fungistatic and fungicidal activity of patulin *in vitro* against mycelium and spores (chlamydospores) of *Ustilago Tritici* (Pers) Rostr. has been determined. The phytocidal effect of patulin on the wheat embryo was elucidated and the activity of patulin against the loose smut of wheat *in situ* was also investigated. Furthermore, the inactivity of patulin in wheat kernel was discussed and the theory to explain the inactivity of patulin in the scutellum of wheat kernel was outlined.

It was found that addition of 25 ml./l of potato extract to the modified Czapek-Dox medium stimulated the germination of the conidia of *Penicillium* and enhanced the formation of surface pellicle, thus shortening the time of incubation for maximum production of antibiotic. The best yield of crystalline patulin was also obtained from this medium which amounted to 1.35 gm. per litre of metabolism solution. On the other hand addition of peptone to modified Czapek-Dox medium reduced the yield of patulin in surface cultures and completely inhibited the formation of antibiotic in submerged cultures.

The 1 : 20,000 dilution of patulin reduced the growth *in vitro* of *Ascochyta pinodes*, *A. pisi*, *Epidermophyton floccosum*, *Microsporum lanosum*, *Trichophyton crateriforme*, *T. gypsum* and completely suppressed the growth of *Ustilago Tritici* in 1 : 100,000 dilution.

The fungistatic and fungicidal activity of patulin against mycelium of loose smut of wheat was found to be 1 : 400,000 and 1 : 100,000 respectively. The spores (chlamydospores) of this fungus proved to be more resistant than mycelium and the highest dilution which completely inhibited the germination of spores in the direct contact with the antibiotic proved to be 1 : 66,000 dilution and 1 : 20,000 dilution was required in the pre-treatment of the spores for 24 hours to achieve the same results.

The patulin was found to be a phytocidal to the wheat embryo. Thus when seeds were soaked for 24 hours in 1 : 4,000, 1 : 8,000 and 1 : 20,000 patulin dilutions and were planted in greenhouse soil immediately after



treatment, the germination was reduced by 53.06, 14.39 and 3.06% respectively, if the percentage of germinated seeds in controls (soaked in tap water) was taken as a 100% germination. The number of germinated seeds was further reduced if the seeds, after the treatment, were allowed to dry at room temperature to the original weight. In this case the reduction of germination amounted to 53.61, 16.58 and 4.12% respectively.

Patulin proved to be ineffective against the pathogen *in situ*. Thus when artificially infected seeds were soaked for 24 hours in 1 : 20,000 patulin solution the percentage of infected plants was not reduced as compared with untreated seed material.

The addition of 10 gm. of whole wheat flour to 50 ml. of 1 : 20,000 patulin solution reduced the activity of the solution by four-fifths of the original, and addition of vitamin B<sub>1</sub>, 1.9  $\gamma$ /ml. completely inactivated it. Furthermore, it was found that patulin is not soluble in wheat germ oil.

It was suggested that due to the high concentration of vitamin B<sub>1</sub> and fat lipoids in the scutellum it acts as a protective body for the mycelium of the pathogen imbedded in it.

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#### REFERENCES

1. ANSLOW, W. K., H. RAISTRICK, and G. SMITH. Antifungal substances from moulds. Part I. Patulin (anhydro-3-hydroxymethylene-tetrahydro-1 : 4-pyrone-2-carboxylic acid) a metabolic product of *Penicillium patulum* Bainier and *Penicillium expansum* (Link) Thom. J. Soc. Chem. Ind. 62 : 236-238. 1943.
2. BERGEL, F., A. L. MORRISON, A. R. MOSS, R. KLEIN, H. RINDERKNECHT, and J. L. WARD. An antibacterial substance from *Aspergillus clavatus* and *Penicillium claviforme* and its probable identity with patulin. Nature, 152 : 750. 1943.
3. BERGEL, F., A. L. MORRISON, A. R. MOSS, and H. RINDERKNECHT. An antibacterial substance from *Aspergillus clavatus*. J. Chem. Soc. p. 415-421. Sept. 1944.
4. CHAIN, E., H. W. FLOREY, and M. A. JENNINGS. An antibacterial substance produced by *Penicillium claviforme*. Brit. J. Exp. Path., 23 : 202-205. 1942.
5. CHAIN, E., H. W. FLOREY, and M. A. JENNINGS. Identity of patulin and claviformin. Lancet, 246 : 112-114. 1944.
6. CAVALLITO, C. J., and J. H. BAILEY. Preliminary note on the inactivation of antibiotics. Science, 100 : 390. 1944.
7. FLOREY, H. W., M. A. JENNINGS, and F. J. PHILPOT. Claviformin from *Aspergillus giganteus* Wehm. Nature, 153 : 139. 1944.
8. GEIGER, W. B., and J. E. CONN. The mechanism of the antibiotic action of clavacin and penicilic acid. Jour. Amer. Chem. Soc., 67 : 112-116. 1945.
9. HERRICK, J. A. Antifungal properties of clavacin. Proc. Soc. Exp. Biol. Med., 59 : 41-42. 1945.
10. HINTON, J. J. C. The chemistry of wheat germ with particular reference to the scutellum. Biochem. J., 38 : 214-217. 1944.
11. HOOPER, I. R., H. W. ANDERSON, P. SKELL, and H. E. CARTER. The identity of clavacin with patulin. Science, 99 : 16. 1944.
12. KARROW, E. D., and J. W. FOSTER. An antibiotic substance from species of *Gymnoascus* and *Penicillium*. Science, 99 : 265-266. 1944.

13. KATZMAN, P. A., E. E. HAYS, C. K. CAIN, J. J. VAN WYK, F. J. REITHEL, S. A. THAYER, E. A. DOISY, W. L. GABY, C. J. CARROLL, R. D. MUIR, L. R. JONES, and N. J. WADE. Clavacin, an antibiotic substance from *Aspergillus clavatus*. J. Biol. Chem. 154 : 475-486. 1944.
14. KENT, J. and N. G. HEATLEY. Antibiotics from moulds. Nature, 156 : 295-296. 1945.
15. LOCHHEAD, A. G., F. E. CHASE, and G. B. LANDERKIN. Production of claviformin by soil *Penicillia*. Can. J. Res. E. 24 : 1-9. 1946.
16. RAISTRICK, H., J. H. BIRKINSHAW, S. E. MICHAEL, A. BRACKEN, W. E. GYE, W. A. HOPKINS, and M. GREENWOOD. Patulin in the common cold. Collaborative research on a derivative of *Penicillium patulum* Banier. Lancet, 245 : 625-635. 1943.
17. REILLY, C., A. SCHATZ, and S. A. WAKSMAN. Antifungal properties of antibiotic substances. J. Bact., 49 : 585-594. 1945.
18. ROBBINS, W. J., and K. C. HAMMER. Effect of potato extract on growth of *Phycomyces*. Bot. Gaz., 101 : 912-927. 1940.
19. SCHMIDT, W. H. and A. J. MOYER. Penicillin. I. Methods of assay. J. Bact., 47 : 199-208. 1944.
20. SIMMONDS, P. M. Detection of the loose smut fungi in embryos of barley and wheat. Sci. Agr., 26 : 51-58. 1946.
21. STANSFELD, J. M., A. E. FRANCES, and C. H. HARRIS. Laboratory and clinical trials of patulin. Lancet, 247 : 370-372. 1944.
22. TIMONIN, M. I., and W. D. McFARLANE. Unpublished results.
23. WAKSMAN, S. A. and E. BUGIE. Action of antibiotic substances upon *Ceratostomella ulmi*. Proc. Soc. Exper. Biol. and Med., 54 : 79-82. 1943.
24. WAKSMAN, S. A., E. S. HORNING, and E. L. SPENCER. Production of antibacterial substances fumigacin and clavacin. Science, 96 : 202-203. 1942.
25. ZALESSKY, V. A new method of aneroobiosis in the control of *Ustilago tritici* on wheat. (In Russian). Plant Protection, Leningrad, 1 : 135-138. 1935.



# A NOTE ON SPRING FROST INJURY TO CEREAL CROPS<sup>1</sup>

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Spring frost of unprecedented proportions was encountered at Winnipeg during the period from May 10 to 15. It was unprecedented both in severity and duration. The following is the temperature record at The University of Manitoba.

| <i>Date</i> | <i>Minimum temperature</i><br>° F. |
|-------------|------------------------------------|
| May 10      | 23                                 |
| May 11      | 13                                 |
| May 12      | 16                                 |
| May 13      | 24                                 |
| May 14      | 32                                 |
| May 15      | 17                                 |

The superficial damage to wheat, barley and oats according to observations made May 12 was somewhat as follows:

*Montcalm barley.* All plants completely collapsed to soil level.

*Mindum wheat.* Damage obviously less than in the case of barley. While the barley leaves were wilted and white throughout their area, those of wheat, although frozen back to the soil level, were green some distance back from their tips, and about 8% of the plants showed little or no damage.

*Renown wheat.* Situation similar to that in Mindum wheat, although more of the leaf showed complete collapse and no plants were undamaged.

*Vanguard oats.* Damage decidedly greater than in case of Renown wheat. Plants were not quite as far advanced as those of the other three crops. They were 2 to 4 inches tall (stretched) while those of wheat and barley were 3 to 5 inches. Renown wheat was sown April 12 and 13; Vanguard oats April 24. Mindum wheat and Montcalm barley were sown April 19.

The Mindum wheat and Montcalm barley were on adjoining fields. Renown wheat and Vanguard oats were on fields about one-and-one-half miles distant, and adjoined each other.

In order to obtain quantitative measurements of damage some detailed data were taken beginning May 12. The plan was to determine:

1. The recovery on the basis of plant counts taken immediately after the frost, and repeated at an interval or intervals of several days.
2. A laboratory study of individual plants to determine to what extent, if any, "growing points" or tiller buds might be uninjured behind the line of frost damage.

## RECOVERY AS MEASURED BY PLANT COUNTS

Counts were made in 4 1-rod sections of drill rows in each of the four crops on May 12 and again on May 22. The data are presented in Table 1.

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TABLE 1.—AVERAGE OF PLANT COUNTS IN 4-ROD ROWS OF EACH OF 4 CROPS IMMEDIATELY AFTER SEVERE FROST, AND AT AN INTERVAL OF 10 DAYS

| Crop            | May 15 | May 22      |                      | Reduction<br>in stand |
|-----------------|--------|-------------|----------------------|-----------------------|
|                 |        | Live plants | Dead<br>plants found |                       |
|                 |        |             |                      | %                     |
| Montcalm barley | 116    | 89          | 7                    | 23                    |
| Mindum wheat    | 78     | 67          | 3                    | 14                    |
| Renown wheat    | 259    | 219         | 7                    | 15                    |
| Vanguard oats   | 123    | 98          | 12                   | 20                    |

According to these data barley and oats made distinctly less recovery than Mindum wheat or Renown wheat. The recovery of barley and oats was essentially the same, and that of the 2 wheats essentially the same. The recovery may be said to have been reasonably good in all cases since the greatest damage left 77% of the original stand. The yield from such stand will probably not be significantly reduced.

On May 22 Mindum and Renown wheat had the appearance of a full stand. Mindum showed a healthy green colour. The discoloration of the original damaged leaves was practically completely obscured by the younger leaves that had completely overgrown them. In the Renown wheat some discoloration was still in evidence.

Montcalm barley also had the superficial appearance of a full stand. It presented a pale green aspect because the original whitened damaged leaves were still in evidence among the newer green ones.

Vanguard oats gave the impression of a somewhat reduced stand although it appeared good.

The leaves of barley and wheat were now 6 to 8 inches long when stretched. Those of oats were 4 to 6 inches.

#### LABORATORY STUDY OF PLANTS

Representative plants were taken up and brought to the laboratory on May 15. These were carefully examined under hand lenses and low power binoculars to determine;

- How far back frost damage extended.
- Whether there was a live "shoot" within the enveloping damaged leaves or leaf sheaths behind the damage line.
- Whether live tiller shoots or tiller buds could be found.

The following statements describe the typical situation on the basis of examination of 15 to 25 plants from each crop:

*Mindum and Renown wheat.* In nearly all cases the culm, or a core of inner leaves enveloping the culm was found undamaged at some distance behind the line of obvious damage. One or two tillers starting just above the kernel and varying in length from  $\frac{1}{16}$  to one inch long were found invariably. The most common length was less than  $\frac{1}{2}$  inch.

*Montcalm barley.* The situation was similar to that in the case of wheat. In most cases the line of obvious damage was further toward the base of the plant, being somewhat below the soil level.





FIG. 1. Frost damaged barley plants dissected to show uninjured internal and basal "shoots". At left: central culm. At right: central culm and two tillers (about two thirds natural size).

Fig. 1 shows two typical barley plants. On the one at the left the short undamaged culm is shown, the outer envelope of leaves and leaf sheaths having been dissected away. The plant at the right shows the main culm and two tillers.

*Vanguard oats.* In most cases an undamaged central core was found some distance back from the line of damage. In a few plants—about one-fifth of those examined—the tip of this central core had been damaged by frost. Tillers or distinctly discernible tiller buds were not found in any of the plants.

#### DROUGHT COMPLICATION

The damage situation may have been influenced more or less by drought. No rain fell during a period from prior to sowing up to the time of the final observations. The moisture situation at the time of sowing was satisfactory. Observations made on June 1 showed that there had been no further consequential reduction in stand in Mindum or Renown wheat. A very decided reduction in the stand of Montcalm barley was due very largely if not entirely to cutworms. The oat plot, where the earlier observations had been made, had been plowed up and resown because it appeared to be suffering from drought. Since it may be assumed that rain would have promoted recovery, and good recovery was made in spite of the absence of rain, it may be concluded that wheat, oats and barley, at least the varieties involved in this study, will withstand 19 degrees of frost, when the plants are in the stage represented by those in this study, namely 3 to 5 inches tall with 2 to 4 leaves unfolded.

# TESTING SEED FOR SMUT SPORES AS AN AID IN CONTROLLING CEREAL SMUTS IN SASKATCHEWAN<sup>1</sup>

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The use of the centrifuge method for examining cereal seed for the presence of smut spores, as a means of deciding whether or not the seed needs to be treated for the control of smut, is receiving increasing attention throughout the Prairie Provinces at the present time. However, the idea has already been subjected to a considerable amount of investigation and trial in the Province of Saskatchewan. As early as 1935, Simmonds and Mead (8) suggested that the centrifuge method could be used to determine whether or not a given sample of wheat seed needed treatment for bunt. More recently, Russell and Ledingham (6) and Mead *et al.* (4) tested a large number of cereal seed samples produced in Saskatchewan. These samples were given comprehensive tests for the presence of physical abnormalities and pathogenic organisms, including smut spores. In the first of these two papers, the authors reported the results of field experiments with wheat seed which had been tested for smut and they suggested that the centrifuge test should be used before deciding whether or not to treat for smut. In both papers, the data presented showed that cereal seed produced in this province is relatively free from appreciable amounts of smut and other pathogenic fungi. Greaney and Machacek (2), after examining cereal seed from all over Canada, stated that, "The cleanest wheat seed examined in 1939 was from Saskatchewan."

Since it has been found that much of the cereal seed produced in Saskatchewan is free from smut, the farmers in this area have been advised to have their seed tested for the presence of smut spores before deciding whether or not to treat it. However, this laboratory was neither staffed nor equipped to give this service to the farmers on a large scale. Therefore, in response to the need, a commercial laboratory in Saskatoon commenced testing cereal seed for smut, using the method developed in our laboratory. It has now been in operation for 6 years and, during that time, has tested thousands of samples of seed wheat and hundreds of samples of oats and barley. During the past 4 years, a large number of samples from this commercial laboratory have been sown in field plots to find out how the amount of smut on the seed compared with the amount of infection in the resultant crop grown under normal field conditions. Also, the records of the laboratory have been synopsized to show what percentages of the samples tested fell into the different smut classes, and how the incidence of the smut diseases compared in the different soil zones of the province. This paper embodies the results of these investigations.

## MATERIALS AND METHODS

Samples of wheat, oats, and barley were sown in field plots at Saskatoon for 4 years in succession. In the last year, duplicate plots of oats and barley were sown at Indian Head. For seed, an equal number of samples

<sup>1</sup> Contribution No. 859 from the Division of Botany and Plant Pathology, Science Service, Dominion Department of Agriculture, Ottawa, Canada.

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was taken at random from each smut class of each cereal. These varied from samples which were entirely free from smut spores to those which carried a heavy spore load. The amount of smut on the seed, indicated by the various smut classes, is explained by Russell and Ledingham (6, p. 673). A few rows were sown with artificially smutted seed each year for the purpose of comparison.

Sufficient seed was picked by hand from each sample to sow 5 8-foot rows with 100 seeds each. This hand-picking, although a laborious process, got rid of a lot of weed seeds, trash, and poor seed and insured much better germination. The seed was sown in regular order, starting with the cleanest samples and working up to the most heavily smutted, in order to avoid contaminating the cleaner samples during seeding. The rows were sown 6 inches apart as in ordinary field practice. The wheat in these tests was sown relatively early; i.e., about the first of May, but the barley and oats were sown about the twentieth of May, because numerous tests conducted here and elsewhere have shown that bunt is favoured by cool soil temperatures, while on the other hand the covered smuts of oats and barley are favoured by warmer temperatures at seeding time. No soil temperature data were taken at seed level but at a depth of 6 inches the average difference in mean temperature for the 10-year period, 1927 to 1937, at Saskatoon on May 1 and May 21 was 5° C. According to records supplied by the Physics Department of the University of Saskatchewan, the average mean temperature of the air for a 44-year period at Saskatoon on these two dates varied by 5° C.

At harvest time, the heads of smut in the barley and oats were counted in the field because they could be seen easily and this method reduced the amount of labour involved. As bunt of wheat is sometimes hard to detect, especially if only a few kernels in a head are affected, the rows of wheat were cut and tied in small sheaves, and later they were taken to the laboratory and threshed. This was accomplished by scrubbing the heads with a metal block, covered with wire screening, as they lay on a sheet of screening ( $\frac{1}{4}$ -inch mesh) over white cardboard. By this method, even a single bunt ball in a head could be detected readily. During the first 3 years, only the smutted heads were counted but in the fourth year the healthy heads were counted also, in order that the exact percentages of infection could be computed.

At the beginning of the tests, the loose smuts of wheat and barley were not taken into consideration because it was known that they could not be controlled by treating the seed with formaldehyde or fungicidal dusts. After it was found that the false loose smut of barley was present in this area, the heads of loose smut occurring in the rows of barley were recorded separately and an investigation of the loose smuts of barley was begun. However, the figures given in Table 1 for barley smut refer only to covered smut, caused by *Ustilago Hordei* (Pers.) Lagerh.

In these experiments, in the first 2 years, the 3 "trace" classes were not kept separate and are all listed under "trace." Later, however, it was realized that it was important to separate these classes in order to ascertain which classes carried enough smut to justify seed treatment. Accordingly, they were not only kept separate during the last 2 years of the test, but twice as many samples were sown as in each of the other 4 classes.

EXPERIMENTAL RESULTS

The data secured from the 4 tests are summarized in Table 1. The results obtained were what one would expect except that there were, in some cases, great variations in the amount of smut produced by different seed samples in the same smut class. Little or no smut appeared in the

TABLE 1.—THE AVERAGE PERCENTAGE OF SMUTTED HEADS DEVELOPED FROM SEED OF EACH SMUT CLASS, SHOWING THE RELATION BETWEEN SPORE LOAD AND SUBSEQUENT INFECTION UNDER ORDINARY FIELD CONDITIONS

| Year | Cereal | Total number of samples | Percentages of smut occurring in the various classes |         |       |         |        |          |       |
|------|--------|-------------------------|--|---------|-------|---------|--------|----------|-------|
|      |        |                         | Clean  | Trace — | Trace | Trace + | Slight | Moderate | Heavy |
|      |        |                         | %  | %       | %     | %       | %      | %        | %     |
| 1942 | Wheat  | 20                      | 0  | —       | 0.10  | —       | 0.17   | 0        | 0.77  |
|      | Oats   | 20                      | 0  | —       | 0.33  | —       | 1.60   | 5.17     | 7.50  |
|      | Barley | 20                      | 0  | —       | 0.10  | —       | 0.17   | 1.47     | 1.87  |
| 1943 | Wheat  | 50                      | 0  | —       | 0.16  | —       | 0.95   | 0.80     | 1.60  |
|      | Oats   | 50                      | 0  | —       | 0     | —       | 0      | 0        | 0.24  |
|      | Barley | 50                      | 0  | —       | 0     | —       | 0      | 0        | 0.03  |
| 1944 | Wheat  | 100                     | 0.05   | 0.01    | 0.22  | 0.56    | 1.17   | 1.16     | 1.67  |
|      | Oats   | 100                     | 0.01   | 0.04    | 0.05  | 0.17    | 0.36   | 0.69     | 2.85  |
|      | Barley | 100                     | 0.01   | 0.04    | 0.05  | 0.06    | 0.08   | 0.19     | 0.23  |
| 1945 | Wheat  | 100                     | 0  | 0.03    | 0.17  | 0.33    | 0.96   | 0.80     | 2.00  |
|      | Oats   | 200                     | 0.14   | 0.09    | 0.07  | 0.65    | 1.05   | 5.15     | 8.85  |
|      | Barley | 200                     | 0.01   | 0.05    | 0.09  | 0.10    | 0.55   | 1.22     | 3.75  |

rows from seed graded as clean and, on the whole, there was a significant increase in the amount of infection from seed of the successively higher smut classes. In the case of wheat, even the heavily naturally smutted seed never produced as much bunt as artificially inoculated seed sown in the same tests. There were some instances in which the plants of a class had less smut than those of the class below it. As examples of this, the wheat in the "moderate" class produced less bunt every year than the wheat in the "slight" class. Also, in any one class there were, in some cases, great variations in the amount of smut produced by different samples. In all classes, there were usually a number of samples which produced no smut whatsoever. These were more numerous in the lower classes and

TABLE 2.—THE NUMBER OF SAMPLES IN EACH CLASS WHICH FAILED TO PRODUCE ANY SMUT. THE FIGURES ARE BASED ON THE FIELD TESTS OF 1944 AND 1945

| Cereal | Smut classes |      |         |      |       |      |        |      |        |      |          |      |       |      |
|--------|--------------|------|---------|------|-------|------|--------|------|--------|------|----------|------|-------|------|
|        | Clean        |      | Trace — |      | Trace |      | Trace† |      | Slight |      | Moderate |      | Heavy |      |
|        | 1944         | 1945 | 1944    | 1945 | 1944  | 1945 | 1944   | 1945 | 1944   | 1945 | 1944     | 1945 | 1944  | 1945 |
|        |              |      |         |      |       |      |        |      |        |      |          |      |       |      |
| Wheat  | 9            | 10   | 18      | 17   | 10    | 15   | 6      | 14   | 4      | 3    | 1        | 2    | 1     | 1    |
| Oats   | 9            | 8    | 18      | 12   | 15    | 13   | 10     | 6    | 1      | 1    | 3        | 0    | 0     | 0    |
| Barley | 9            | 9    | 16      | 12   | 17    | 13   | 14     | 11   | 7      | 2    | 5        | 1    | 2     | 0    |

Note:—20 samples were used in each of the trace classes and 10 samples in each of the other classes.



fewer in the higher ones, as is shown in Table 2. In the more heavily smutted classes, oats sometimes produced a considerably higher percentage of smutted heads than did wheat or barley.

These results, shown in Table 1, suggest that, in ordinary farm practice, the samples in the classes with a "trace" or less of smut on the seed could have been sown without treatment, as in no case was the average infection for these classes over one-quarter of 1%. However, more experimental data are needed to determine this point. Certain individual samples in these lower classes showed as high as 1.2% infection in the case of wheat, 1.4% in the case of oats, and 0.7% in the case of barley, but very few of the samples had infections approaching these amounts. On the other hand, certain samples bearing a "trace plus" or more of smut produced considerable smut in the subsequent crop, indicating that seed grain bearing a "trace plus" or more should be treated.

### ANALYSIS OF SEED-TESTING RECORDS

Six years' records of the commercial laboratory's tests have been studied and the data relating to smut have been summarized. The two main objects of the study were to learn what proportions of the cereal seed fell in the different smut classes from year to year, and how the incidence of the covered smuts varied in the three main soil zones of Saskatchewan.

The data showing the distribution of seed samples over the smut classes are given in Table 3. For the first 3 years, the 3 "trace" classes

TABLE 3.—THE PERCENTAGES OF WHEAT, OATS, AND BARLEY FALLING IN THE VARIOUS SMUT CLASSES FROM 1939 TO 1944, INCLUSIVE

| Cereal | Crop year | Smut classes |        |       |        |        |          |       |
|--------|-----------|--------------|--------|-------|--------|--------|----------|-------|
|        |           | Clean        | Trace— | Trace | Trace+ | Slight | Moderate | Heavy |
|        |           | %            | %      | %     | %      | %      | %        | %     |
| Wheat  | 1939      | 74.1         | —      | 25.2  | —      | 0.7    | 0.0      | 0.0   |
|        | 1940      | 85.1         | —      | 13.4  | —      | 0.7    | 0.5      | 0.3   |
|        | 1941      | 84.9         | —      | 13.4  | —      | 0.6    | 0.8      | 0.3   |
|        | 1942      | 76.1         | 10.0   | 7.9   | 2.7    | 2.1    | 0.9      | 0.4   |
|        | 1943      | 79.6         | 8.5    | 6.3   | 2.1    | 2.0    | 1.3      | 0.2   |
|        | 1944      | 83.1         | 5.1    | 6.6   | 2.2    | 1.9    | 0.8      | 0.1   |
|        | Mean      | 80.5         | 7.9    | 6.9   | 2.3    | 1.3    | 0.7      | 0.2   |
| Oats   | 1939      | 35.6         | —      | 52.5  | —      | 7.8    | 2.9      | 1.2   |
|        | 1940      | 31.2         | —      | 38.3  | —      | 16.7   | 9.3      | 4.5   |
|        | 1941      | 26.4         | —      | 43.4  | —      | 14.6   | 8.1      | 7.5   |
|        | 1942      | 13.0         | 25.6   | 15.1  | 9.8    | 17.2   | 11.9     | 7.4   |
|        | 1943      | 27.5         | 17.3   | 19.5  | 15.0   | 10.7   | 7.1      | 2.8   |
|        | 1944      | 39.5         | 11.3   | 14.2  | 18.8   | 10.7   | 4.2      | 1.1   |
|        | Mean      | 28.9         | 18.1   | 16.3  | 14.5   | 12.9   | 7.2      | 4.1   |
| Barley | 1939      | 22.2         | —      | 55.5  | —      | 6.1    | 16.2     | 0.0   |
|        | 1940      | 17.7         | —      | 39.8  | —      | 16.8   | 17.7     | 8.0   |
|        | 1941      | 16.5         | —      | 49.5  | —      | 13.7   | 13.7     | 6.6   |
|        | 1942      | 7.8          | 16.2   | 23.6  | 21.8   | 14.0   | 9.6      | 7.0   |
|        | 1943      | 17.9         | 19.5   | 20.8  | 17.8   | 13.9   | 6.8      | 3.3   |
|        | 1944      | 23.7         | 10.3   | 13.7  | 24.4   | 17.1   | 5.5      | 5.5   |
|        | Mean      | 17.6         | 15.3   | 19.4  | 21.3   | 12.6   | 11.6     | 5.1   |

were not kept separate, but during the last 3 years this was done. A number of points brought out by these data may be emphasized. In the first place, a large percentage of the wheat samples are free or practically free from bunt spores. If it is considered that all wheat samples found to be free of bunt do not require treatment, then the average percentage of the samples, tested from the years 1939 to 1944, inclusive, which did not require treatment was 80.5%. In the case of oats and barley, a smaller percentage of the seed samples were free from smut. The figure for oats, corresponding to the figure given above for wheat, was 28.9% and for barley it was 17.6%. This represents a very substantial portion of the wheat seed and a considerable portion of the oat and barley seed of Saskatchewan. In the relatively wet season of 1942, the covered smuts of oats and barley were unusually plentiful. This is reflected in the low percentage of these cereals in the "clean" class that year. Although there appear to be distinct seasonal variations in the smut spore load on the seed samples tested, there is no clear-cut indication of either an increase or a decrease in the prevalence of the covered smuts throughout the period from 1939 to 1944.

The influence of regional differences should be taken into consideration when studying the smut problem as a whole. This effect can be seen even in a single province (See Table 4). Most of the land which has been brought under cultivation in Saskatchewan lies in the first 3 soil zones as set forth by Mitchell *et al.* (5). These are referred to as the Brown, Dark

TABLE 4.—VARIATIONS IN THE PERCENTAGES OF SAMPLES REQUIRING TREATMENT IN THE DIFFERENT SOIL ZONES

| Cereal | Year | Number of samples tested |            |       | Percentage needing treatment <sup>1</sup> |            |       |
|--------|------|--------------------------|------------|-------|---|------------|-------|
|        |      | Brown                    | Dark brown | Black | Brown                                     | Dark brown | Black |
| Wheat  | 1942 | 557                      | 773        | 492   | 13.1                                      | 3.8        | 1.6   |
|        | 1943 | 593                      | 883        | 711   | 14.7                                      | 3.4        | 1.0   |
|        | 1944 | 847                      | 1461       | 1095  | 10.8                                      | 4.2        | 1.5   |
|        | Mean | —                        | —          | —     | 12.9                                      | 3.8        | 1.4   |
|        |      |                          |            |       |   |            |       |
| Oats   | 1942 | 59                       | 198        | 210   | 37.3                                      | 45.4       | 50.5  |
|        | 1943 | 85                       | 213        | 249   | 16.5                                      | 36.1       | 40.1  |
|        | 1944 | 73                       | 260        | 295   | 12.3                                      | 37.6       | 38.9  |
|        | Mean | —                        | —          | —     | 22.0                                      | 39.7       | 43.2  |
|        |      |                          |            |       |   |            |       |
| Barley | 1942 | 36                       | 83         | 104   | 50.0                                      | 50.6       | 55.8  |
|        | 1943 | 49                       | 132        | 116   | 22.4                                      | 48.4       | 43.1  |
|        | 1944 | 59                       | 99         | 95    | 28.8                                      | 59.6       | 60.1  |
|        | Mean | —                        | —          | —     | 33.7                                      | 52.9       | 53.0  |
|        |      |                          |            |       |   |            |       |

<sup>1</sup> Samples showing a "trace plus" or more of smut.

Brown, and the Black soil zones respectively because of the prevailing colour of the surface soil. The climate and vegetation vary somewhat from zone to zone, and these factors have, over a long period of time, left their imprint on the soils found therein. The records of the commercial

laboratory were analysed and the percentages of the samples of each cereal, for the 3 soil zones comprising most of the cultivated areas of the province, were listed under their appropriate smut classes. In Table 4 is shown for each zone the percentage of samples which required treatment for smut. Figures for the first 3 years are not given because the "trace" classes were not kept separate in those years.

It is evident from the data presented in Table 4 that there is a distinct difference in the prevalence of the cereal smuts in the different zones. Bunt of wheat is most prevalent in the Brown zone and least prevalent in the Black, while the reverse appears to be true of the covered smuts of oats and barley. However, the conclusions concerning the smuts of barley and oats are based on much fewer samples than those respecting bunt of wheat.

### DISCUSSION

Any distinct change in common agricultural methods is apt to be accompanied by a period of uncertainty and adjustment. When it was first suggested that the farmers of this province should depart from the old practice of treating their seed grain regularly each year and treat it in future only when tests indicated that treatment would be beneficial, many farmers and other agricultural men were slow to accept the proposal. They wanted to be shown that the tests were reliable and the results satisfactory.

The investigations dealt with in this paper were designed to secure information as to the practicability of the new method of attacking the smut problem. It was felt that the method should be tested under field conditions over a period of years. This has been done. The evidence accumulated during the 4 years that these field tests have been conducted, shows that the new method is reliable and practicable as far as the covered smuts of wheat, oats, and barley are concerned. The value of the method depends upon a number of factors as indicated below.

In the first place this method will prove most useful in areas, such as Saskatchewan, where a large proportion of the seed grain is relatively free from smut and other seed-borne parasites. In regions where the proportion of seed samples which need treatment is high, there is not the same incentive to have the seed tested because the chances are great that it will require treatment. Also in regions where wheat is sown in the autumn and exposed to infection in the soil, testing the seed for smut will not serve the same purpose. In the case of wheat in Saskatchewan, for the past 6 years, over 80% of the seed tested by the commercial laboratory was free from bunt spores and, presumably, fit to sow without treating. This would represent a big saving in labour and material to the farmers of this province if the new practice were adopted on a large scale. In the case of oats and barley, a larger proportion of the seed required treatment but a considerable proportion of the seed of these cereals could have been sown to advantage without treatment. While the material used in these experiments may not have been fully representative of all the cereal seed used in those years in Saskatchewan, the number of samples was fairly large and fairly well distributed over the Province (See Table 4).



To some, it may seem surprising that different samples of seed, bearing approximately the same load of smut spores, should vary so much in the amount of smut produced under the same environmental conditions in the field. However, as far as oats and barley are concerned, Zade (10), Gage (1), and Tapke (9) have shown that the mycelial inoculum established under the hulls is of more importance in determining the amount of infection in the subsequent crop than is the amount of ungerminated spores clinging to the outside of the hulls. But it is the latter form of inoculum that is detected by the centrifuge test, so that it cannot be expected that equal infections will always develop from different seed samples from the same smut class. It seems probable that the samples which bore considerable smut spores but produced no smut when sown in the field had little or no smut mycelium established beneath the seed hulls.

According to the authors just mentioned, the extent to which smut mycelium develops beneath the hulls of the coarse grains depends upon a number of factors operative from flowering time through to and including the storage period. Moisture is one of the main factors favouring the development of the mycelium. In Saskatchewan conditions are, on the average, more moist in the Black soil zone than in the Brown soil zone, and therefore more favourable to the development of this type of smut inoculum. It seems quite probable that this is the main reason why the covered smuts of oats and barley are more prevalent in the Black soil zone.

It has been suggested that the same amount of smut spores on cereal seed might produce heavier infections of smut if the seed were sown in certain other parts of Saskatchewan. For example, it is possible that higher percentages of bunt would develop in the Brown soil zone and higher percentages of the covered smuts of oats and barley would develop in the Black soil zone than were obtained at Saskatoon, which lies in the Dark Brown soil zone. However, in 1945 the barley and oat plots were duplicated at Indian Head, which lies in the Black soil zone, and the infections in that case were lower than those obtained at Saskatoon. More experimental data is needed to settle this point.

It should be recognized that other considerations may render it advisable to apply treatment to seed grain even when smut is absent. The presence of other seed-borne diseases, such as seedling blight caused by *Helminthosporium sativum* P. K. & B., in some cases may make treatment desirable. Although *H. sativum* causes a discoloration of wheat and barley seed, known as smudge, and severely damages seedlings developing from affected kernels, it is seldom present on more than 1% of the kernels on the average in this province (4, 6, 7). Also, seed-coat damage from threshing and handling when the grain is dry and brittle may call for seed treatment as a protection against the saprophytic as well as parasitic organisms of the soil (4, 6). On the other hand, extensive experiments with sound seed, reported by Greaney and Machacek (2, 3), and experiments conducted at this laboratory with plump wheat seed having a low percentage of embryo exposure (4, 6), have shown that seed treatment in such cases gives little or no improvement in yield. So that, unless there is an actual need for seed treatment, it may be merely a waste of time and money to apply it. In fact, treatment with formaldehyde is a detriment rather than a help, in some cases, because it reduces the germinative vigour of the seed.

Whenever possible, it is desirable to have cereal seed tested by an approved method for the presence of seed-borne diseases. The prime objective of seed-testing, from the pathological standpoint, is not to discourage the application of seed treatment but to ascertain which samples will benefit from it and which samples do not require it. The objective may be considered two-fold, to emphasize its desirability in those cases where it is needed and to prevent economic waste where it is apt to do no good. If conducted on a large scale, this practice should lead to a more thorough and efficient control of cereal diseases. In order to be completely satisfactory, seed testing should determine the germinative vigour of the sample, the condition with regard to seed-borne parasites, and the condition with regard to mechanical injury of the seed coat. The records of such tests, if conducted on a large scale over a period of years, would yield valuable information as to the prevalence and distribution of such diseases throughout the region from which the samples were obtained.

One aspect of the problem, which should be kept in mind, is the attitude of the farmers to this new way of keeping the cereal smuts under control. In the majority of cases, the ones who have tried it seem to be very well satisfied with it. The results have served to give them confidence in the recommendations which they have received concerning the samples they have submitted for testing. When their seed does not require treatment, this information gives them great satisfaction, and when treatment is advised they are not likely to neglect the operation.

There is little doubt that the new method of securing control of the cereal smuts is workable in Saskatchewan as far as bunt of wheat is concerned; but in the case of barley and oats, there may be more room for argument. To begin with, less study and investigation have been devoted to the seed of the coarse grains in this province; and it has been shown that a smaller proportion of the samples examined were free of smut spores. Also, it may be found that seed-borne parasites other than smuts are more prevalent and important in the coarse grains than they are in wheat. The presence of false loose smut of barley (*Ustilago nigra* Tapke) complicates the problem, as far as making recommendations is concerned. On account of the loose nature of the spore masses, most of the spores are blown away before the grain is threshed and fewer spores are left to be detected by the centrifuge test, and yet a considerable amount of inoculum in the form of mycelium may be established in the parenchyma of the hulls and in the epidermis of the pericarp, as described by Gage (1) and Zade (10) in the case of the loose smut of oats. In such cases, seed treatment should be recommended, because the fungicides used in the control of covered smut (*Ustilago Hordei* (Pers.) Lagerh.) will effectively destroy this fungus too, but owing to the early dissipation of the spores the test may not indicate that an appreciable amount of inoculum is present. Further investigation is needed to clarify the question.

#### SUMMARY

A new method of dealing with the covered smuts of cereals has been developed in Saskatchewan. The seed samples are examined by the centrifuge method, and only those samples which carry an appreciable amount of smut spores are treated.

The method has been in operation on a fairly large scale for 6 years. It has given good results and appears to be regarded favourably by the farmers who have tried it. Its reliability has been checked by growing a large number of tested samples in experimental field plots.

Field experiments indicate that it is safe in this province to sow without fungicidal treatment seed grain which shows no more than a trace of smut, providing that it is free from other important seed-borne parasites and that it is of good quality, but more experimental data is needed to determine the proper limits of tolerance.

There is a considerable variation in the amount of smut which will develop, under the same growing conditions, in the crop from different samples carrying equal spore loads of smut. In other words, the amount of smut which will develop under the same environmental conditions appears to depend on the variety of the host, the physiologic form of the fungus, and other factors, as well as upon the amount of inoculum present. In the case of oats and barley, the chief factor may be the extent to which the mycelium of the parasite has become established under the hulls of the grain.

An analysis of 6 years' records of seed testing showed that approximately 80% of the wheat, 30% of the oats, and 20% of the barley which was tested was free from smut spores and therefore did not require treatment for smut.

There appears to be a distinct correlation between the soil zone and the prevalence of the cereal smuts in Saskatchewan. Bunt of wheat was most prevalent in the Brown soil zone and least prevalent in the Black, while the reverse was true of the covered smuts of oats and barley.

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#### REFERENCES

1. GAGE, G. R. Studies of the life history of *Ustilago Avenae* (Pers.) Jensen and of *Ustilago levis* (Kell. & Swing.) Magn. Cornell Univ. Agr. Exp. Station Memoir 109. 1926.
2. GREANEY, F. J., and J. E. MACHACEK. The prevalence of seed-borne fungi on cereals in certain seed inspection districts of Canada. Sci. Agr. 22 : 419-437. 1942.
3. GREANEY, F. J., and J. E. MACHACEK. The prevalence and control of seed-borne diseases of cereals in Manitoba. Sci. Agr. 26 : 59-79. 1946.
4. MEAD, H. W., R. C. RUSSELL, and R. J. LEDINGHAM. The examination of cereal seeds for disease and studies on embryo exposure in wheat. Sci. Agr. 23 : 27-40. 1942.
5. MITCHELL, J., H. C. MOSS, and J. S. CLAYTON. Soil survey of southern Saskatchewan. Univ. of Sask. Soil Survey Rept. No. 12. 1944.
6. RUSSELL, R. C., and R. J. LEDINGHAM. Wheat seed testing from the pathological standpoint with special reference to embryo exposure. Sci. Agr. 21 : 761-775. 1941.
7. RUSSELL, R. C. The relative importance from the pathological standpoint of two types of smudge on wheat kernels. Sci. Agr. 23 : 365-375. 1943.
8. SIMMONDS, P. M., and H. W. MEAD. The examination of wheat seed to determine the disease factor. Sci. Agr. 16 : 175-179. 1933.
9. TAPKE, V. F. Studies on the natural inoculation of seed barley with covered smut (*Ustilago Hordei*). Jour. Agr. Res. 60 : 787-810. 1940.
10. ZADE, A. Neure Untersuchungen uber die Lebensweise und Bekampfung des Haferflugbrandes (*Ustilago Avenae* (Pers.) Jens.). Angew. Bot. 6 : 113-125. 1924.



# DRIED WHEY AS A SUBSTITUTE FOR DRIED BUTTERMILK IN CHICK RATIONS<sup>1</sup>

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Dried buttermilk and dried skimmilk were, some years ago, among the main sources of riboflavin in poultry rations. During the war years, as these products became increasingly difficult to obtain, it was timely that part of the great volume of liquid whey, available from the increased production of cheese and casein, should be conserved in the form of dried whey to aid in offsetting this deficiency.

Most of the work reported on the use of dried whey has been in connection with its value in production and hatching rations. Davis, Norris and Heuser (5) reported that hatchability in poultry was favourably influenced by the inclusion of dried whey in the ration. They attributed the beneficial effect of the dried whey to its riboflavin content rather than its protein content. In addition, Christiansen, Halpin and Hart (2), in hatchability studies, showed by results obtained from groups receiving synthetic riboflavin, that the factor supplied by dried whey was riboflavin. Bearse (1) found that a breeder's ration, fed with scratch grain, gave an equal increase in hatchability when supplemented with 5% powdered whey, 3 pounds of condensed buttermilk per 100 birds per day or 3% condensed buttermilk containing cereal grasses.

Culton and Bird (4) conducted experiments with chicks, studying growth and "curled-toe paralysis" as affected by supplying varying amounts of riboflavin as crystalline riboflavin, dried skimmilk and dried whey. They concluded that the growth promoting effect of dried whey could be explained on the basis of its riboflavin content, but that dried skimmilk exerts a greater growth promoting effect than can be explained on this basis. Christiansen *et al* (2) tested dried whey as a substitute for soybean oil meal in chick rations and concluded that the protein of whey (milk albumin) was without value as a supplement, and that the supplementary value of whey was due to its vitamin content. Jukes (10) and Sullivan *et al* (12, 13) showed, on the other hand, that the growth response from whey powder could not be accounted for by its riboflavin content alone.

Hammond and Titus (8) reported that dried whey was adequate in chick rations as a source of the members of the vitamin B-G complex ordinarily supplied by dried skimmilk. Dried whey substituted for dried skimmilk in a poor quality ration consisting chiefly of ground oats and dried skimmilk, gave superior results. Hill and co-workers (9) suggested that improvement in the growth of chicks resulting from the addition of 10% dried distillers' solubles or 5% dried brewer's yeast or 4% dried whey to either of two practical chick rations was not due to riboflavin or protein but to an unidentified growth factor or factors.

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From the work which has been reported it would appear therefore, that, although the main value of dried whey in poultry feeding lies in its vitamin content and more especially its riboflavin content, there is a possibility that other factors play a part in its promotion of growth.

### EXPERIMENTAL

In order to determine the suitability of dried whey as a substitute for part or all of the dried buttermilk in rations for growing chicks, eleven groups, each of 35 Barred Plymouth Rock cockerel chicks, were fed on rations containing from 0 to 20% of dried whey<sup>1</sup> replacing successively larger amounts of dried buttermilk.

The newly hatched chicks were banded and placed in electrically heated brooders in an air conditioned room kept at a thermostatically controlled temperature of 80° F. At the end of 4 weeks the chicks were sufficiently well feathered to allow the use of the electric heaters to be discontinued, and at the end of 5 weeks the condition of the birds warranted their being placed in growing batteries where no artificial heat was supplied.

Feed and water were before the birds at all times and the total consumption of feed for the 10-week period per group was recorded. One per cent each of oyster shell, bone meal and grit were mixed in the ration for the first 6 weeks after which time these ingredients were omitted from the ration and supplied *ad libitum*.

Birds which died during the first week of the experiment were replaced with birds from the same hatch which had been maintained on a normal chick starter ration. Deaths occurring during the initial week were not considered as being attributable to the ration. The mortality during the subsequent 9 weeks was recorded.

The birds were weighed individually at the end of the second week and at the end of each subsequent week until they were 10 weeks of age, at which time the experiment was terminated.

Dried whey was used to replace dried buttermilk in the rations on the basis that it contained approximately one-half as much riboflavin as found in dried buttermilk. This assumption was based on the results of analysis conducted in this laboratory on a large number of commercial samples during recent years together with the results of other workers. Calculation of the riboflavin content of each of the experimental rations showed that there was an adequate amount, at least, of riboflavin in all of the rations regardless of the combination of dried whey and dried buttermilk. Using the lowest assay figures ever obtained in these laboratories, Evans, Young and Branion (6, 7), for the various types of feedstuffs incorporated in the ration, the minimum riboflavin levels of the rations ranged from 1450 to 1800 micrograms per pound. Calculations of the maximum levels, using the highest assay figures on record in these laboratories, showed a range of riboflavin values of 2600 to 3500 micrograms per pound. Since it is extremely unlikely that all of the ration components were of minimum riboflavin potency, there is little doubt that all of the rations met the

<sup>1</sup> The dried whey was obtained from the Milverton Creamery, Milverton, Ontario. It was stated to contain no filler.

recommended allowance for riboflavin of about 1600 micrograms per pound (Norris (11), Titus (14) and Committee on Animal Nutrition, National Research Council, Washington (3) ).

The protein level was maintained in all rations at approximately 18%. To this end, it was necessary to increase the amount of meat meal slightly at the expense of the ground barley as increasing amounts of dried whey were used. Analyses showed that the dried whey contained 12.5% crude protein whereas the dried buttermilk contained 31.6%.

The rations used in the experiment are shown in Table 1.

TABLE 1.—COMPOSITION OF RATIONS

| Ingredients                                     | Group 1 | Group 2 | Group 3 | Group 4 | Group 5 | Group 6 | Group 7 | Group 8 | Group 9 | Group 10 | Group 11 |
|---|---------|---------|---------|---------|---------|---------|---------|---------|---------|----------|----------|
| Ground oat groats                               | 21      | 21      | 21      | 21      | 21      | 21      | 21      | 21      | 21      | 21       | 21       |
| Ground wheat                                    | 21      | 21      | 21      | 21      | 21      | 21      | 21      | 21      | 21      | 21       | 21       |
| Ground yellow corn                              | 21      | 21      | 21      | 21      | 21      | 21      | 21      | 21      | 21      | 21       | 21       |
| Cereal grass                                    | 1       | 1       | 1       | 1       | 1       | 1       | 1       | 1       | 1       | 1        | 1        |
| Dehydrated alfalfa <sup>1</sup>                 | 2.5     | 2.5     | 2.5     | 2.5     | 2.5     | 2.5     | 2.5     | 2.5     | 2.5     | 2.5      | 2.5      |
| Salt (iodized and manganized) <sup>2</sup>      | 0.5     | 0.5     | 0.5     | 0.5     | 0.5     | 0.5     | 0.5     | 0.5     | 0.5     | 0.5      | 0.5      |
| Cod liver oil (fortified Nopco XX) <sup>3</sup> | 0.25    | 0.25    | 0.25    | 0.25    | 0.25    | 0.25    | 0.25    | 0.25    | 0.25    | 0.25     | 0.25     |
| Ground barley                                   | 14.50   | 12.75   | 11.75   | 10.25   | 8.75    | 7.25    | 5.75    | 4.50    | 2.75    | 1.25     | 0.00     |
| Dried buttermilk                                | 9       | 8       | 7       | 6       | 5       | 4       | 3       | 2       | 1       | 0        | 0        |
| Dried whey                                      | 0       | 2       | 4       | 6       | 8       | 10      | 12      | 14      | 16      | 18       | 20       |
| Meat meal <sup>4</sup>                          | 9.5     | 10.0    | 10.0    | 10.5    | 11.0    | 11.5    | 12.0    | 12.5    | 13.0    | 13.5     | 13.5     |
| Per cent crude protein <sup>5</sup>             | 18.3    | 18.3    | 18.1    | 18.0    | 18.0    | 18.0    | 18.0    | 18.0    | 18.0    | 18.0     | 18.1     |

<sup>1</sup> A mixture of 3 commercial varieties of dehydrated alfalfa.

<sup>2</sup> This salt contained 0.02% KI and 2.0%  $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$ .

<sup>3</sup> The cod liver oil contained 3000 I.U. Vitamin A and 400 A.O.A.C. units Vitamin D per gram.

<sup>4</sup> A mixture of 3 commercial varieties of meat meal.

<sup>5</sup> Calculated from results of analyses carried out in the Department of Animal Nutrition laboratories.

## RESULTS AND DISCUSSION

The average weekly weights and survival data are shown in Table 2 and the average weekly weights and gains are presented graphically in Figure 1.

The data were compared by the use of the following formulae:

$$(1) \text{ Variance} = \frac{n\sum(x^2) - (\sum x)^2}{n(n-1)}$$

where  $n$  = number of birds per group

$x$  = final weight of each single bird at the end of the 10-week period.

(2) Standard error of the Difference between Means

$$= \sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}$$

where  $s_1^2$ ,  $s_2^2$  and  $n_1$  and  $n_2$  are the variances and the number of chicks respectively for the two groups under comparison.



TABLE 2.—AVERAGE WEEKLY WEIGHTS (GMS.) AND SURVIVAL DATA

|                     | Group | 2 weeks | 3 weeks | 4 weeks | 5 weeks | 6 weeks | 7 weeks | 8 weeks | 9 weeks | 10 weeks |
|---------------------|-------|---------|---------|---------|---------|---------|---------|---------|---------|----------|
| No dried whey       | 1     | 84.6    | 132.8   | 202.2   | 282.2   | 382.1   | 510.0   | 642.5   | 831.1   | 1002.3   |
| 9% dried buttermilk |       | 35*     | 35      | 35      | 35      | 35      | 35      | 35      | 35      | 35       |
| 2% dried whey       | 2     | 93.2    | 151.7   | 226.7   | 332.3   | 447.5   | 583.6   | 773.7   | 921.5   | 1079.7   |
| 8% dried buttermilk |       | 35      | 35      | 35      | 35      | 35      | 34      | 33      | 32      | 32       |
| 4% dried whey       | 3     | 92.5    | 149.3   | 228.1   | 330.2   | 448.8   | 576.9   | 734.0   | 897.4   | 1055.4   |
| 7% dried buttermilk |       | 35      | 35      | 35      | 35      | 35      | 35      | 35      | 35      | 35       |
| 6% dried whey       | 4     | 91.0    | 143.7   | 217.1   | 317.2   | 432.3   | 567.2   | 722.6   | 877.7   | 1025.6   |
| 6% dried buttermilk |       | 35      | 35      | 35      | 35      | 35      | 35      | 35      | 35      | 35       |
| 8% dried whey       | 5     | 88.5    | 142.5   | 219.7   | 319.8   | 433.6   | 574.7   | 718.8   | 898.0   | 1069.5   |
| 5% dried buttermilk |       | 35      | 35      | 35      | 35      | 35      | 35      | 35      | 35      | 35       |
| 10% dried whey      | 6     | 89.9    | 142.6   | 212.6   | 304.8   | 415.1   | 524.3   | 666.0   | 809.7   | 967.2    |
| 4% dried buttermilk |       | 34      | 34      | 34      | 34      | 34      | 34      | 34      | 34      | 34       |
| 12% dried whey      | 7     | 93.9    | 145.2   | 218.1   | 310.7   | 424.9   | 558.7   | 696.1   | 856.8   | 1018.8   |
| 3% dried buttermilk |       | 35      | 35      | 35      | 35      | 35      | 35      | 35      | 35      | 35       |
| 14% dried whey      | 8     | 84.2    | 133.9   | 199.1   | 283.2   | 388.4   | 512.1   | 643.6   | 817.8   | 982.9    |
| 2% dried buttermilk |       | 35      | 35      | 35      | 35      | 35      | 35      | 35      | 34      | 35       |
| 10% dried whey      | 9     | 83.6    | 131.5   | 195.9   | 283.2   | 387.4   | 519.3   | 661.5   | 820.5   | 941.5    |
| 1% dried buttermilk |       | 34      | 34      | 34      | 34      | 34      | 34      | 33      | 33      | 33       |
| 18% dried whey      | 10    | 96.6    | 150.2   | 226.1   | 321.9   | 432.6   | 568.7   | 711.5   | 867.5   | 993.3    |
| No dried buttermilk |       | 35      | 35      | 35      | 34      | 34      | 34      | 34      | 34      | 34       |
| 20% dried whey      | 11    | 86.6    | 136.0   | 202.3   | 286.9   | 387.7   | 511.3   | 621.1   | 782.5   | 888.7    |
| No dried buttermilk |       | 35      | 35      | 35      | 35      | 35      | 35      | 35      | 35      | 35       |

\* Number of survivors.

TABLE 3.—SIGNIFICANCE OF DIFFERENCE BETWEEN RATIONS

| Group number | Significantly better than | Significantly poorer than  |
|--------------|---------------------------|----------------------------|
| 1            | 11                        | 2                          |
| 2            | 1, 6, 8, 9, 10, 11        | None                       |
| 3            | 6, 9, 11                  | None                       |
| 4            | 9, 11                     | None                       |
| 5            | 6, 9, 11                  | None                       |
| 6            | 11                        | 2, 3, 5                    |
| 7            | 11                        | None                       |
| 8            | 11                        | 2                          |
| 9            | None                      | 2, 3, 4, 5                 |
| 10           | 11                        | 2                          |
| 11           | None                      | 1, 2, 3, 4, 5, 6, 7, 8, 10 |

For any group to show a mean weekly weight significantly different from that of another group with a degree of certainty of 95% or greater, the difference between these means must exceed twice the standard error of this difference.

On the basis of the individual weights at 10 weeks, each group was compared with every other group. The results of these comparisons are shown in Table 3.

TABLE 4.—TOTAL FEED CONSUMPTION BY GROUPS FOR THE 10-WEEK PERIOD

| Group No. | Feed (lb.) | Group No. | Feed (lb.) | Group No. | Feed (lb.) |
|-----------|------------|-----------|------------|-----------|------------|
| 1         | 293        | 5         | 290        | 9         | 297        |
| 2         | 293        | 6         | 295        | 10        | 293        |
| 3         | 290        | 7         | 296        | 11        | 294        |
| 4         | 295        | 8         | 294        |           |            |

The feed consumption of each group is given in Table 4. It will be seen that this was fairly constant for all groups and the differences in growth were apparently not related to differences in the amount of feed consumed.

During the initial period of the experiment all groups receiving more than 12% of dried whey showed marked symptoms of oedema. In practically all birds of these groups, general swelling of a watery nature was apparent, indicating increased subcutaneous fluid. The condition appeared during the first week and persisted for 3 to 4 weeks. After this time the external symptoms gradually disappeared. Detailed water consumption records were not kept during the course of the experiment but it was noted that as the amount of dried whey in the ration increased, the water consumption increased. The oedema may have been caused by the large amount of lactose present in the whey. By increasing their water intake the birds may have been able to dilute the excess lactose and to gradually stimulate their excretory system in such a way as to get rid of the excess lactose and at least overcome the external symptoms of the oedematous condition. It will be noted from the survival data (Table 2) that the oedema caused very little, if any, mortality. The growth data in Figure 1 and Table 2, also indicate that the early growth of all groups as judged by weight with the possible exception of that group receiving 20% dried whey, was quite satisfactory, suggesting that, during the period when the symptoms of oedema were evident, no serious loss or retardation of growth occurred. The growth of the 20% group (Group No. 11) was much poorer than that of any other group during the fourth week and this may have been due to the oedema. In the case of the other groups on the high levels of whey, it is possible that a retardation of growth may have occurred but that it was masked by the increase in body water content. In so far as the authors are aware, no other investigators have fed higher levels than 10% of dried whey in chick rations.

From these data it appears that dried whey can be used satisfactorily, in amounts up to 8 or 10%, to replace dried buttermilk in chick rations when the necessary adjustments for riboflavin and protein are made. It should be pointed out, in view of the variation in the commercial processing of dried whey, that these levels may only apply to this particular type of whey. With other types it is possible that either somewhat lower or higher levels would be found to be suitable. In amounts greater than these levels, even up to 18%, growth was still fairly satisfactory, but in view of the development of oedema, it is considered that these higher levels are not advisable. About 2% dried whey appears to give optimum growth while 20%, representing a complete replacement of the dried buttermilk, resulted in definite retardation of growth.

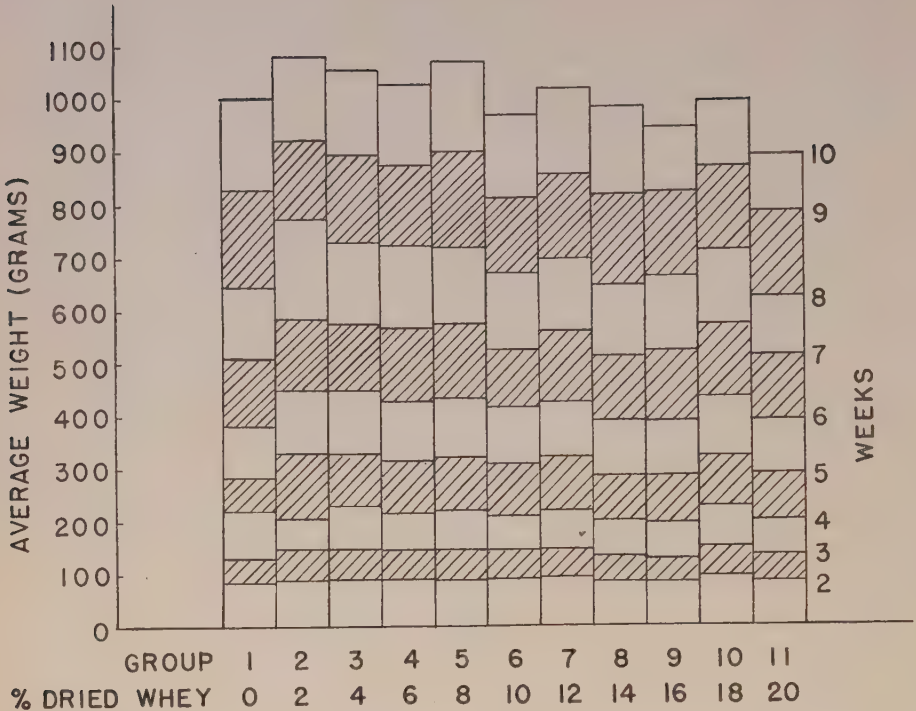


FIGURE 1. Average weekly gains and weights on rations with various amounts of dried whey substituted for dried buttermilk.

The present experiment suggests that dried buttermilk may possess some essential growth factor or factors not present in dried whey. Similarly there also is an indication that dried whey may contain some factor or factors not present in the dried buttermilk. From a consideration of the trend of the results for those groups receiving the "low buttermilk" diets, it would appear that some dried buttermilk, possibly 2 to 3%, may be essential for optimum growth. It also appears, in considering group 1 in relation to the groups receiving the mixture of dried buttermilk and dried whey that some dried whey produces better growth than rations containing dried buttermilk alone.

### CONCLUSIONS

Dried whey, in amounts up to 8 or 10%, was used satisfactorily in rations for growing chicks to replace part of the dried buttermilk when the necessary adjustments were made in the riboflavin and protein levels of the ration.

Higher levels of dried whey, 12 to 20%, resulted in the onset of an oedematous condition, although growth, as judged by weight and mortality, was little, if any, affected. This condition was accompanied by an increased water consumption and the external symptoms gradually disappeared.

Although the quantity of dried buttermilk necessary for good growth appears to be small, there is some indication in this work that it should not be completely replaced by dried whey. There is also a suggestion that dried whey may contain some essential growth factor or factors not present in dried buttermilk.



## REFERENCES

1. BEARSE, G. E. A comparison of molasses, whey, condensed buttermilk and condensed buttermilk containing cereal grasses as supplements to a breeding hen ration. *Poul. Sci.* 17 : 433-434. 1938.
2. CHRISTIANSEN, J. B., J. G. HALPIN, and E. B. HART. Studies on the nature of the effective supplements for soybean oil meal in rations for the production of hatching eggs. *Poul. Sci.* 19 : 55-60. 1940.
3. COMMITTEE ON ANIMAL NUTRITION, NATIONAL RESEARCH COUNCIL, WASHINGTON, D.C. Recommended nutrient allowances for poultry. 1944.
4. CULTON, T. G., and H. R. BIRD. The effect of some riboflavin supplements in chick growth and curled-toe paralysis. *Poul. Sci.* 19 : 347. 1940.
5. DAVIS, H. J., L. C. NORRIS, and G. F. HEUSER. Further evidence on the amount of vitamin G required for reproduction in poultry. *Poul. Sci.* 17 : 87-93. 1938.
6. EVANS, E. V., D. M. YOUNG, and H. D. BRANION. Riboflavin content of milk products for feeding purposes. *Sci. Agr.* 24 : 510-515. 1944.
7. EVANS, E. V., D. M. YOUNG, and H. D. BRANION. Riboflavin content of Canadian feedstuffs. *Sci. Agr.* 25 : 542-545. 1945.
8. HAMMOND, J. C., and H. W. TITUS. Substitutes for dried skim milk in the diet of growing chicks. *Poul. Sci.* 22 : 411-414. 1943.
9. HILL, F. W., M. L. SCOTT, L. C. NORRIS, and G. F. HEUSER. Deficiency of unidentified vitamins in practical chick rations. *Poul. Sci.* 23 : 253-255. 1944.
10. JUKES, T. H. Biological assay of lactoflavin with chicks. *Journ. Nut.* 14 : 223-233. 1937.
11. NORRIS, L. C. Vitamin requirements of poultry. *Poultry Tribune*, February, 1942.
12. SULLIVAN, R. A., E. REEVES, E. BLOOM, and W. RATEIKE. The value of dairy products in nutrition. 1. Basal rations for chick assays. *Poul. Sci.* 21 : 387-395. 1942.
13. SULLIVAN, R. A., E. BLOOM, E. REEVES, and W. RATEIKE. The value of dairy products in nutrition. 2. Growth factors for chicks. *Poul. Sci.* 21 : 396-406. 1942.
14. TITUS, H. W. Practical nutritive requirements of poultry. *Food and Life*, U.S. Dept. Agric. Yearbook, 1939.

# ALFALFA SEED PRODUCTION IN NORTHERN SASKATCHEWAN AS AFFECTED BY BEES, WITH A REPORT ON MEANS OF INCREASING THE POPULATIONS OF NATIVE BEES<sup>1</sup>

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## INTRODUCTION

In the province of Saskatchewan the principal area growing alfalfa seed is located on the grey soils in the northern districts of settlement. The first alfalfa seed produced in this area was grown about 1930 near the village of White Fox, about 90 miles east of Prince Albert, and this district still is one of the main centres of production. Farmers soon recognized the value of the new crop and the acreage increased rapidly. The estimated production for the Province reached a peak of 5,500,000 pounds in 1941 and the estimated average for the 6 years 1939-44 is 2,400,000 pounds. This seed consists almost entirely of the Grimm variety of *Medicago media* L. Pioneer growers, with small fields surrounded by bush, reported exceptionally heavy yields, up to as high as 1000 pounds per acre and commonly 200 to 500 pounds per acre. However, with the clearing and cultivation of wooded land and a corresponding increase in the size of field, seed yields tended to drop sharply, until at the present time the average yield is about 75 pounds with 200 to 300 being uncommon. Consequently, in later years, the maintenance of seed production has depended largely upon the newer fields, especially those situated in districts recently opened for settlement.

The yields of alfalfa seed are influenced by many factors. Among these, weather conditions, plant disease, and both beneficial and harmful insects are of prime importance. The value of bees as pollinators in the White Fox area, with methods of protecting them, has been reported summarily by Salt (65). Extensive investigations have been carried out by Knowles (37), mainly upon the relations of climate and bees to tripping. The investigations outlined in the present paper form not only a continuation of their work but also a more intensive study of the bee populations and of the means of maintaining them.

## LIST OF BEES TAKEN AT WHITE FOX, WITH TAXONOMIC NOTES

Early in this study it became apparent that bees varied both generically and specifically in their ability to trip alfalfa blossoms and that the principal genera concerned were *Megachile* and *Bombus*, while *Osmia*, *Coelioxys*, *Psithyrus* and *Anthophora* were of value. Bees belonging to the genera *Halictus* (s.l.) and *Andrena* have been reported as trippers in southern Ontario by Engelbert (18) but no members of either of these genera were seed tripping alfalfa in northern Saskatchewan. As far as possible, the individual species were studied, fairly extensive collections being made and identified. The list of species which follows includes all the bees collected on various flowers in the White Fox area. Although some species were not collected on alfalfa, the list is made complete to facilitate later reference in this paper and to aid in distribution studies.

*Megachilidae*

*Osmia dacotensis* Mitch.

*Megachile* (*Anthemiois*) *relativa* Cress.

*Megachile* (*Anthemiois*) *nivalis* Friese

*Megachile* (*Anthemiois*) *inermis* Prov.

*Megachile* (*Delomegachile*) *gemula* Cress.

*Megachile* (*Delomegachile*) *gemula* var. *fulvogemula* Mitch.

*Megachile* (*Delomegachile*) *melanophaea* Sm.



*Megachile (Delomegachile) frigida* Sm. (= *vidua* Sm.)

*Megachile (Xanthosarus) latimanus* Say

*Megachile (Sayapis) pugnata* Say

*Coelioxys lucrosa* Cress.

*Coelioxys moesta* Cress.

*Coelioxys dubitata* Sm. (= *rufitarsis* Sm.)

### *Bombidae*

*Bombus rufocinctus* Cress.

*Bombus rufocinctus* var. *iridis* Ckll. and Porter

*Bombus rufocinctus* var. *prunellae* Ckll.

*Bombus rufocinctus* var. *albertensis* Ckll.

*Bombus borealis* Kby.

*Bombus californicus* Sm.

*Bombus terricola* Kby.

*Bombus ternarius* Say

*Bombus ternarius* var. *expallidus* Ckll.

*Bombus huntii* Greene

*Bombus perplexus* Cress.

*Bombus vagans* Sm.

*Bombus vagans* subsp. *bolsteri* Franklin

*Psithyrus ashtoni* Cress.

*Psithyrus suckleyi* Greene

*Psithyrus insularis* Sm.

### *Anthophoridae*

*Anthophora furcata* Pz.

### *Apidae*

*Apis mellifera* L.

Although subspecies and varieties are included in the above list as a matter of record, in all subsequent discussion such subdivisions are grouped under the species name.

It has been suggested by Mitchell (46) that *nivalis* may possibly be a race of *relativa* or a species closely related to *relativa*. The males of the two forms appear similar, although the males of *Megachile* normally show specific differences in the genitalia and concealed sternites (48-50). The females differ in the colour of the scopa (entirely pale in *relativa*; apically black in *nivalis*), in the pubescence of the sixth tergite (tomentose and golden in *relativa*; fuscous in *nivalis*), and in the greater average size of *nivalis*. Females in the subgenus *Delomegachile* are sometimes subject to variation of the scopal colour within specific limits (50).

Material in the Canadian National Collection amply supports these views. However, the females bear on the sixth tergite some suberect hairs; in *relativa* these are small, pale and evenly distributed but in *nivalis* they are large, black and widely separated, occurring laterally. At White Fox 10 males were reared from the same log as 7 *nivalis* females, one pair being from the same tunnel. These males were compared with 10 males, believed to be *relativa* and obtained from scattered localities in eastern Canada, where *nivalis* is rare; no stable differences were found, although in each case the genitalia and hidden sternites were examined.

The distribution of the two forms is of interest in deciding their taxonomic status. Field collecting at White Fox, which is well within the Canadian Zone, showed *relativa* to be somewhat more abundant than *nivalis*; 35 individuals as against 21. However, *relativa* becomes rare further north, and *nivalis* common; conversely, *relativa* is common further south, while *nivalis* is rarely found in southern Canada or in the United States, except along the Rocky Mountains.

According to Mitchell (49), *relativa* is to be found from "North Carolina west to New Mexico and California and northward throughout Canada to Mackenzie and Newfoundland"; his records, however, include only 3 localities from northern Canada (Slave Lake, Alta., and Fort Simpson and Fort Providence in Mackenzie). At hand in the Canadian National Collection there are 122 Canadian specimens of *relativa* females, of which only 4 can be considered as at all northern (Athabasca, Bear L., Shaftesbury and Smoky River Crossing, Alta.; all collected by E. H. Strickland between August 18 and 24, 1915); moreover, these localities are still within the Canadian Zone, in which *relativa* is common.

Published records of Mitchell (48, 49, 50) show that *nivalis* is almost entirely restricted to northern Canada with a southward extension along the Rocky Mountains, the holotype being taken at the 9000 ft. level on Pikes Peak, Colo. Exceptional localities are Lake Nipigon, Ont., Montreal, P.Q. and Vancouver, B.C. (47), and Maine (51). Additional Canadian records of *nivalis* females are Cameron Bay, Great Bear Lake (July 6 to 27, 1937, T. N. Freeman); Tazin River (July 12, 1914, F. Harper); Fort Wrigley, Mackenzie River (July 20 to 25, 1922, C. H. Crickmay); Lesser Slave Lake, Alta. (August 17, 1915, J. M. Swaine); Jasper Park, Alta. (September, 1916, F. Johansen); Waterton, Alta. (August 14, 1921, E. H. Strickland); Vernon, B.C. (August 2, 1923, D. G. Gillespie) and Gaspé, P.Q. (August 24, 1932, E. B. Watson). The comparative rarity of *nivalis* in southern and eastern Canada is emphasized by the vastly greater collecting that has been carried out in this area than in the north.

At White Fox the visits of these bees to certain flowers were observed (Table 4). Fireweed attracted 19 females of *relativa* and only two of *nivalis*; almost all *relativa* specimens were taken on one of two successive days; the catch for each day was taken at a different locality and at each a single specimen of *nivalis* was taken. Sow thistle attracted 7 females of *relativa* but none of *nivalis*, all records being taken in the same day. As the total collections in 1944 for the White Fox area were 35 for *relativa* and 21 for *nivalis*, the figures for these visits suggest that the two forms have definite flower preferences and are specifically distinct.

The nesting habits may be used in evaluating these two forms, although the various species of leaf-cutter bees may not be entirely restricted to one type of nesting site, such as earth, hollow plant stems or logs. According to Guignard (34), *relativa* (*brevis* Cr.) nests in sunflower stems, although this species has also been reared from a tunnel in a bank (Hicks, 35). At White Fox 10 females of *nivalis* were reared to the adult stage, but not one of *relativa* (Table 5). It is perhaps also notable that the nests of *nivalis* (and *inermis*) were uniformly blocky and neat, made from poplar leaves pressed closely together. A series of three joined nests with three associated

*relativa* females are at hand; these nests are narrower, irregularly shaped and made apparently with poplar leaves inside and rose leaves outside. The apices of the rose leaves project untidily from the sides of the nests. The original site of the *relativa* nest is not known.

These biological data, showing apparent differences in nest-building and in flower preferences, are not sufficiently numerous to allow *nivalis* to be considered without doubt as specifically distinct. However, the two forms are treated so, as a matter of caution and convenience.

Among the species reported by Knowles (37) on alfalfa was *M. montivaga*. The specimens, however, have proved to be *M. relativa*. The name *frigida*, too, should be substituted for that of *vidua*, as used in the papers of Knowles (37) and Mitchell (49), since the name *frigida* has priority (Mitchell, 51). Similarly, in the genus *Coelioxys* the name *dubitata* has priority over *rufitarsis*, since Viereck (73) appears to be the first reviser. The identity of the male of *Coelioxys moesta* is discussed under the heading "Parasites and Predators."

### THE VALUE OF VARIOUS BEES IN TRIPPING

#### GATHERING OF STATISTICAL DATA

The amount of tripping and the seed yield of alfalfa at White Fox have been correlated by Knowles (37) with the numbers of bumble bees, of leaf-cutter bees and honey bees. Knowles' work was continued during 1943 by the junior author and during 1944 by both authors.

During 1943, 33 fields were sampled between July 14 and August 11, while, during 1944, 30 fields were examined between July 20 and August 1. The density of the bee populations in each field was determined by counting the number of bumble bees, leaf-cutter bees and honey bees found in 40 squares, each approximately 8 feet square and 10 to 20 yards apart. No attempt was made to recognize the different species. While bumble bees are active at lower temperatures than either leaf-cutter bees or honey bees, yet no records were taken with the temperature below 67° F., when the leaf-cutter bees were inactive in the field; consequently the estimations of the total activity of the bumble bees are low.

The amount of flower-tripping was estimated by counting the number of freshly tripped flowers on 200 racemes, 5 being taken from each of the 40 sampling squares. These racemes were average in size and were picked at random within the square. A flower was considered as freshly tripped only until the banner petal began to fold. According to Tysdal (72) this occurs about 2 hours after tripping, so that records were not taken until the temperature had been above 67° F. for at least 2 hours, thus ensuring that the activities of the leaf-cutter bees would be fully recorded.

The comparative yield of seed from each field was found by averaging the yield from 20 plots, each a square yard in area and chosen at random.

The various correlations between the different factors are shown in Table 1, while the numbers of each group of bees in relation to the number of tripped flowers are summarized in Table 2. These figures are discussed below, according to the type of bee.



TABLE 1.—CORRELATION VALUES BASED ON THE NUMBER OF BEES IN 40 SAMPLES, EACH OF ABOUT 64 SQUARE FEET, AND OF TRIPPED FLOWERS ON 200 RACEMES FROM EACH FIELD. SEED YIELDS ESTIMATED FROM 20 SQUARE YARD SAMPLES FROM EACH FIELD

| Type of correlation | Comparison                                     | Year         |              |
|---------------------|--|--------------|--------------|
|                     |  | 1943, N = 33 | 1944, N = 30 |
| Simple              | Tripping vs. total leaf-cutter and bumble bees | .72†         | .61†         |
| Simple              | Tripping vs. leaf-cutter bees                  | .72†         | .57†         |
| Simple              | Tripping vs. bumble bees                       | .53†         | .44†         |
| Simple              | Tripping vs. honey bees                        | .12          | -.05         |
| Simple              | Leaf-cutter vs. bumble bees                    | .36*         | .32          |
| Simple              | Seed yield vs. leaf-cutter bees                | .50†         | —            |
| Partial             | Tripping vs. bumble bees                       | .39*         | .34          |
| Partial             | Tripping vs. leaf-cutter bees                  | .67†         | .51†         |
| Partial             | Leaf-cutter bees vs. bumble bees               | .01          | .07          |
| Multiple            | Tripping vs. all bees                          | .78          | .63†         |

\* Exceeds 5% point.

† Exceeds 1% point.

TABLE 2.—NUMBER OF BEES IN 40 SAMPLES, EACH OF ABOUT 64 SQUARE FEET, AND OF TRIPPED FLOWERS ON 200 RACEMES FROM EACH FIELD

| Year | No. of fields | Av. no. honey bees | Av. no. leaf-cutter bees | Av. no. bumble bees | Av. no. leaf-cutter and bumble bees | Av. no. tripped flowers | Av. no. tripped flowers per wild bee |
|------|---------------|--------------------|--------------------------|---------------------|-------------------------------------|-------------------------|--------------------------------------|
| 1943 | 33            | 13.1               | 3.1                      | 5.2                 | 8.3                                 | 93.1                    | 11.2                                 |
| 1944 | 30            | 7.3                | 3.0                      | 2.7                 | 5.7                                 | 64.9                    | 11.4                                 |

### HONEY BEES AS TRIPPING AGENTS

Honey bees are usually recorded as being of little or no importance as tripping agents in the northwestern portion of North America (Gray, 33; Knowles, 37; Piper, *et al.*, 59; Salt, 65; Sladen, 68; Tysdal, 72). However, in Manitoba, Lejeune and Olson (38) observed that one day 12 honey bees tripped 28% of the 114 flowers visited by them, on the next day 17 bees tripped 17.1% out of 105, and two days later 16 bees tripped 0.0% out of 158; no honey bees were seen tripping during the remainder of the season. A somewhat similar occurrence was reported in New South Wales by Dwyer and Allman (17).

In the White Fox area large numbers of honey bees were observed visiting alfalfa flowers. In most instances the bees approached the flower from the side of the keel, as reported by various other investigators (17, 33, 38, 65). However, a few honey bees were noted which regularly approached the flower between the keel and banner petals and almost invariably tripped the flower. They were slower than wild bees, principally because they usually were trapped by the violent tripping mechanism and had some difficulty in extricating themselves. According to Gray (33), the honey

bee enters from the side of the flower because the tongue is otherwise too short to reach the nectaries of the alfalfa flower. A scarcity of other pollen sources may force honey bees to collect it from alfalfa flowers, which would not ordinarily be visited for this purpose. This might prompt the behaviour of the exceptional individuals noted at White Fox, as well as accounting for the results of Lejeune and Olson (38), and of Dwyer and Allman (17); the latter reported 27.39% of the flowers set pods where a hive of honey bees was caged with relatively few alfalfa plants.

Tripping by honey bees seems to be rare and to be caused by abnormal conditions; these may include the scarcity or absence of preferred pollen and the occurrence of hot and dry weather. The presence of self-tripping plants of alfalfa may also cause records to be incorrectly attributed to honey bees visiting at the time of tripping. Furthermore, honey bees have a strong tendency to visit only one species of plant during a flight and therefore they can appear unduly important.

At present at White Fox honey bees are unimportant as tripping agents, the statistical data obtained supporting the field observations. Knowles (37) reported a significant value of  $+ .54$  between the amount of tripping and the number of honey bees in 1942 but believed that this was caused by the attraction of honey bees and leaf-cutter bees to the same field. As shown in Table 1, correlations of  $+ .12$  and  $- .05$  were obtained in 1943 and 1944, showing the unimportance of honey bees in tripping, even though Table 2 shows that there were more honey bees present than leaf-cutter bees or bumble bees.

Honey bees may be indirectly responsible for a reduction in the number of visits by other bees. It has been suggested by Salt (65) that they rob the flower of its nectar, probably making it less attractive to insects that can pollinize it. This of course would occur only when the alfalfa crop is secreting merely a small amount of honey, either during the cooler parts of the day or else before or after the main bloom.

Furthermore, the honey bee may be detrimental to the setting of alfalfa seed because of the effects of its foraging upon the food supplies of the wild bees. Indeed, Pearson (58) has commented upon the honey bee as follows: "So efficient is it as a collector of pollen and honey and so ubiquitous has it become, there can be no question but what its inroads cause a serious diminution in the food supplies of native bees, particularly in bad seasons." This statement was based upon the author's experience with the bees in Wisconsin, and was not supported by data. Nevertheless, it should be borne in mind that bee-keeping is a common practice in the White Fox district and that Pearson's opinion may be applicable here, at least when pollen and nectar are scarce.

On the other hand, honey bees may be of considerable value in cross-pollinating self-tripping alfalfa plants such as those described by Armstrong and White (2), if a strain of this type were developed. At Saskatoon, honey bees have been observed to visit freely the tripped flowers on these plants. In addition, a preliminary experiment indicated that pollination with foreign pollen one hour after tripping, and possibly longer, will result in cross-fertilization. By the selection of self-sterile, self-tripping strains of plants, tripping would occur independently of insect visitation and cross-pollination might be effected by honey bees.

## WILD BEES AS TRIPPING AGENTS

Wild bees have long been considered as important in the setting of alfalfa seed and are virtually the only group of insects associated with tripping. In the prairie provinces of Canada the value of wild bees has been studied by Sladen (68), Gray (33), Salt (65), Lejeune and Olson (38) and Knowles (37); these workers emphasized the importance of leaf-cutter bees and bumble bees. In the northern part of North America other groups of bees that trip alfalfa belong in the genera *Nomia* (72), *Andrena* (1, 18), *Halictus* (18) and *Anthophora* (68).

At White Fox the importance of bumble bees and leaf-cutter bees has already been pointed out by Knowles (37). This work was continued for two more seasons. The results are presented in Tables 1 and 2 and amply confirm Knowles' conclusions. Both simple and partial correlations suggest that the leaf-cutter bees form the more valuable group. However, high correlations were obtained between bumble bee populations and tripping; furthermore even the partial correlation, where the effects of leaf-cutter bees and honey bees were held constant, was significant in 1943. Tysdal (72) found that bumble bees varied from 38 to 80% in effectiveness of tripping. While our correlation values indicate that leaf-cutter bees are more important than bumble bees, the summary presented in Table 2 suggests that equal value should be attached to each group. The average number of flowers tripped per field in 1943 was considerably greater than in 1944. Leaf-cutter bees were equally abundant in each year but bumble bees were more numerous in 1943 than in 1944. Yet, when the number of tripped flowers is based upon the total number of wild bees present, there is no essential difference in the two years.

In any comparison between the relative value of leaf-cutter bees and bumble bees it should be kept in mind that bumble bees work at lower temperatures and reduced sunlight, and, consequently, for longer periods each day. Had some records been taken at temperatures unfavourable only to leaf-cutter bees, bumble bees would have undoubtedly been shown to be more important than the tables suggest.

On the other hand, bumble bees seem to show considerable variation in population from year to year. In 1942 their numbers in the alfalfa fields at White Fox were considered as very small by Knowles (37); in 1943 they were more numerous in alfalfa fields than leaf-cutter bees and were twice as numerous in 1943 as in 1944 (Table 2). The numbers of bumble bees in red clover, too, have been shown by Morrison (54) to vary excessively from year to year in Quebec.

A large number of observations were made on the tripping abilities of individual bees. The method used was to record the tripping activity of a particular bee, which was then captured, given a number corresponding to its record, and later identified. Notes were taken on the habitat and the behaviour in regard to tripping. In some cases a record was made of the number of alfalfa flowers visited per minute, these data being summarized in Table 3.

Among the species of leaf-cutter bees, *Megachile frigida*, *M. melanophaea*, *M. nivalis*, *M. relativa*, *M. gemula*, *M. inermis* and *M. latimanus* were observed actively tripping alfalfa flowers; as shown in Table 3, all species



TABLE 3.—SUMMARY OF OBSERVATIONS ON THE NUMBER OF ALFALFA FLOWERS VISITED PER MINUTE BY DIFFERENT SPECIES OF BEES<sup>1</sup>

| Species                  | Number of observations | Rate per minute |       |
|--------------------------|------------------------|-----------------|-------|
|                          |                        | Average         | Range |
| <i>Megachile frigida</i> | 28                     | 18              | 10-34 |
| <i>M. melanophaea</i>    | 1                      | 15              | —     |
| <i>M. inermis</i>        | 4                      | 15              | 8-27  |
| <i>M. latimanus</i>      | 5                      | 20              | 16-23 |
| <i>Bombus borealis</i>   | 3                      | 21              | 12-27 |
| <i>B. rufocinctus</i>    | 1                      | 20              | —     |
| <i>B. terricola</i>      | 7                      | 17              | 12-28 |
| <i>B. vagans</i>         | 2                      | 16              | 15-16 |
| <i>B. ternarius</i>      | 3                      | 13              | 10-17 |

<sup>1</sup> *M. nivalis* and *relativa* were also found to be fast and efficient in tripping blooms.

tripped a high percentage of the flowers visited but *M. inermis* (with the exception of one individual) was much slower in its movements than any other species. Although well represented in the general bee population, neither *nivalis* nor *relativa* is included in Table 3. Both species are fast and efficient trippers but are very active and easily frightened so that, although many attempts were made to take records, the bee always disappeared before it had been observed for a full minute. Seventeen records were taken of *M. pugnata* but in no case was it found visiting alfalfa, although it was often collected on other flowers growing in alfalfa fields. This species seems to be of no economic importance to alfalfa seed-setting, especially as Robertson (63) has recorded it as restricted to certain of the Compositae. Very few detailed observations were made of the relative tripping efficiency of males and females of the same species. However, many males of *M. frigida* were taken on alfalfa flowers; several were actively tripping, although in general they were not as efficient as the females and in several instances approached the flower from between the keel and wing petals in the same manner as honey bees, presumably seeking nectar rather than pollen.

The bumble bee species, *Bombus borealis*, *rufocinctus*, *terricola*, *vagans* and *ternarius* visited alfalfa and all were more or less effective as tripping agents (Table 3). *B. huntii* was observed on alfalfa but not tripping, and *perplexus* and *californicus* were seen only on other host plants but not in sufficient numbers to form any opinion as to their flower preference. In addition, the guest bumble bees, *Psithyrus suckleyi* and *ashtoni* were observed tripping. *P. insularis* was collected on alfalfa but was not observed to trip any flowers.

Among other wild bees, *Coelioxys* was observed visiting alfalfa. Two individuals tripped flowers but they did so very slowly and awkwardly and usually were trapped by the tripping mechanism. *Anthophora furcata*, although a common visitor, was only once seen to trip alfalfa flowers; Sladen (68) has also reported *furcata* on alfalfa but only at Ottawa. This

species has a very long tongue and, although it often approaches the flower between the banner and keel petals, it is still able in this position to extract nectar without tripping.

Since the speed at which an individual bee works is an important factor in evaluating its effectiveness as a tripping agent, records were taken of the rate at which bees visited alfalfa flowers. The data, summarized in Table 3, indicate that individual bumble bees and leaf-cutter bees visited alfalfa flowers at about the same rate. The difference in efficiency appears to be in the higher percentage of flowers tripped by the leaf-cutter bees. However, certain individual bumble bees were as equally efficient in tripping as the best of the leaf-cutter bees.

### THE ECOLOGY OF VARIOUS ALFALFA BEES

In any study of the effects of a changing habitat upon an insect population it is necessary for the whole problem to be reviewed, even though only parts can be examined immediately. The present report is therefore preliminary in nature, covering only those phases that could be dealt with best at the present time. However, indications of subordinate problems and suggestions of possible remedies have been included; these are considered as forming an important, and frequently omitted, part of a preliminary report on a problem.

The establishment of alfalfa as a seed crop in northern Saskatchewan has had two important results upon the native bees:—

1. The establishing of this and other crops has increased greatly the amount of raw land that has been broken, thus destroying many bees, much of their native food, and many of their nesting sites.
2. The growing of this seed crop has made an abundant supply of food available to many native bees, including those needed to trip the blossoms. This food, however, is produced only for a part of the season.

Paradoxically, the grower has ravaged the bees upon which he depends for the fertilization of his alfalfa flowers, although producing a temporary abundance of food for them.

The preservation of natural nesting areas adjacent to the alfalfa has already been urged in order to maintain the numbers of bees as trippers (Salt, 65; Tysdal, 72; Knowles, 37) and this is perhaps all that is needed in certain areas. Nevertheless, such a policy may not necessarily maintain the alfalfa bees in satisfactory numbers. As emphasized by Nicholson (56) and Smith (70), the average density of an insect population fundamentally is regulated by the competition for space and food, and by factors such as parasites, predators and disease. These phases should be considered in any attempt to maintain or increase populations of alfalfa bees.

### PLANT HABITATS

Before factors such as space, food and inquilines can be assayed, the number of natural habitats must be noted, as well as their proportions and their value in providing shelter and food. In the White Fox area the

natural vegetation and soil types conform to three general types. By far the greater area is dominated by *Populus tremuloides* Michx.<sup>1</sup> (aspen poplar) and *P. tacamahacca* Mill. (balsam poplar) with an occasional sprinkling of *Picea glauca* (Moench) Voss. (white spruce). With these trees are associated the following shrubs and herbs that probably are sources of pollen and nectar: *Grossularia* spp. (gooseberries), *Ribes* spp. (wild currants), *Distegia involucrata* (Richards.) Cockerell (swamp honeysuckle), *Mertensia paniculata* (Ait.) Don. (bluebell), *Lathyrus* spp. (wild sweet pea), *Vicia americana* Muhl. (wild vetch), *Svida instolonea* A. Nels. (dogwood), *Chamaepericlimenum canadense* (L.) Aschers. & Graebn. (bunchberry), *Fragaria glauca* (S. Wats.) Rydb. (wild strawberry), *Rubus melanolasius* Focke (wild raspberry), *Chamaenerion spicatum* (Lam.) S. F. Gray (fireweed), *Aster* spp. (wild aster) and *Solidago* spp. (goldenrod). *Taraxacum officinale* Weber (dandelion), *Sonchus arvensis* L. (sow thistle) and *Trifolium hydridum* L. (alsike clover) are commonly found in cultivated fields and along roadsides. This general area is characterized by clay and clay loam soils.

Smaller areas throughout the district, composed of light sandy soils, are dominated by *Pinus Banksiana* Lamb. (jackpine). Very few species of plants yielding nectar and pollen are found in this soil type, the only important ones being *Arctostaphylos Uva-ursi* (L.) Spreng. (bearberry), *Vitis-Idaea punctata* Moench. (sand cranberry) and *Cyanococcus canadensis* (Richards.) Rydb. (blueberry). However, a few of the species from the poplar habitat are sparsely represented here too, perhaps because the sandy areas do not cover large acreages and the poplar vegetation is often nearby.

Another very distinct vegetative type occurs on poorly drained peat soils and is commonly known as muskeg or swamp vegetation. The dominant trees are *Larix laricina* (DuRoi) Koch (tamarack) and *Picea Mariana* Mill. B.S.P. (black spruce). Commonly associated flowering plants which may be sources of pollen and nectar are *Betula* spp. (swamp birch), *Ledum groenlandicum* Oeder (Labrador tea), *Andromeda polifolia* L. (bog rosemary), *Kalmia polifolia* Wang. (pale laurel), *Oxycoccus palustris* Pers. (swamp cranberry), *Rubus Chamaemorus* L. (cloudberry) and *Xylosteon caeruleum* (L.) Dum.-Cours. (blue fly honeysuckle). No critical examination was made of this habitat and only a few bees were collected from it. However, it occasionally occurs near alfalfa fields and probably should be considered in any future study.

Wherever possible, collections were classified according to their proximity to the poplar and jackpine habitats. A summary of the data obtained is presented in Table 4, together with data on the flowers visited by individual bees. Plant records excluded individually from this table are as follows:—

*Vicia americana* Muhl. (Wild Peavine): *B. rufocinctus*, *B. vagans*.

*Melilotus alba* Desv. (White sweet clover): *B. ternarius*, *B. rufocinctus*.

*Agoseris* sp., ? *scorzoneraefolia* (Schrad.) Greene: *M. pugnata*.

*Agastache* sp. (Giant hyssop): *B. ternarius*, *B. vagans*.

*Castilleja* sp. (Indian paint brush): *B. californicus*.

<sup>1</sup> These botanical names conform to the usage in Rydberg's Flora of the Prairies and Plains of Central North America. New York Botanical Garden, N.Y. 1932.



TABLE 4.—HOST PLANTS AND HABITAT RELATIONSHIPS OF WILD BEES IN THE WHITE FOX AREA

| Species of wild bee        | Number of records |             |           |          |           |               | Total number of bees observed | Habitat |           |                |
|----------------------------|-------------------|-------------|-----------|----------|-----------|---------------|-------------------------------|---------|-----------|----------------|
|                            | Alfalfa           | Sow thistle | Dandelion | Fireweed | Goldenrod | Other* plants |                               | Poplar  | Jack-pine | Total observed |
| <i>Megachile frigida</i>   | 85                | 3           | 0         | 30       | 0         | 4             | 122                           | 88      | 37        | 125            |
| <i>M. melanophaea</i>      | 4                 | 0           | 0         | 0        | 0         | 0             | 4                             | 2       | 3         | 5              |
| <i>M. gemula</i>           | 2                 | 0           | 0         | 1        | 0         | 0             | 3                             | 1       | 0         | 1              |
| <i>M. nivalis</i>          | 5                 | 0           | 1         | 2        | 0         | 0             | 8                             | 18      | 0         | 18             |
| <i>M. relativa</i>         | 8                 | 7           | 1         | 20       | 0         | 1             | 37                            | 32      | 2         | 34             |
| <i>M. inermis</i>          | 4                 | 9           | 2         | 8        | 0         | 2             | 25                            | 26      | 1         | 27             |
| <i>M. latimanus</i>        | 13                | 3           | 0         | 0        | 0         | 0             | 16                            | 1       | 15        | 16             |
| <i>M. pugnata</i>          | 0                 | 6           | 6         | 0        | 0         | 5             | 17                            | 12      | 3         | 15             |
| <i>Anthophora furcata</i>  | 8                 | 0           | 1         | 0        | 0         | 7             | 16                            | 5       | 0         | 5              |
| <i>Bombus huntii</i>       | 1                 | 0           | 0         | 1        | 0         | 0             | 2                             | 1       | 0         | 1              |
| <i>B. borealis</i>         | 8                 | 2           | 0         | 1        | 0         | 2             | 13                            | 8       | 4         | 12             |
| <i>B. californicus</i>     | 0                 | 0           | 0         | 0        | 0         | 1             | 1                             | 1       | 0         | 1              |
| <i>B. perplexus</i>        | 0                 | 0           | 0         | 2        | 0         | 0             | 2                             | 2       | 0         | 2              |
| <i>B. rufocinctus</i> s.l. | 13                | 10          | 0         | 1        | 0         | 4             | 28                            | 14      | 12        | 26             |
| <i>B. terricola</i>        | 45                | 4           | 1         | 1        | 3†        | 3             | 57                            | 36      | 12        | 48             |
| <i>B. vagans</i> s.l.      | 4                 | 2           | 0         | 5        | 0         | 3             | 14                            | 10      | 2         | 12             |
| <i>B. ternarius</i>        | 16                | 6           | 1         | 5        | 3         | 8             | 39                            | 21      | 5         | 26             |
| <i>Psithyrus ashtoni</i>   | 1                 | 0           | 0         | 0        | 0         | 0             | 1                             | 1       | 0         | 1              |
| <i>P. insularis</i>        | 2                 | 1           | 0         | 0        | 0         | 0             | 3                             | 1       | 0         | 1              |
| <i>P. suckleyi</i>         | 3                 | 1           | 0         | 0        | 0         | 0             | 4                             | 1       | 3         | 4              |
| Totals                     | 222               | 54          | 13        | 77       | 6         | 40            | 412                           | 281     | 99        | 380            |

\* The plants included in this column have been listed previously on p. 398.

† Males.

As shown in Table 4, some species of bees showed a decided preference for distinct habitats. This was not shown as strongly in *Bombus* as in *Megachile*, although the associations of *Bombus* may be masked by their ability to forage far afield. In *Megachile* the species *inermis*, *relativa* and *nivalis* frequented only the poplar habitat and *latimanus* the jackpine areas, while *frigida* was commonly found in both and *melanophaea* infrequently in both. No satisfactory explanation is available for the apparent ecological restriction of some of these bees, although more complete information on their habits of nesting and foraging might clear up the point.

No attempt was made to correlate the sampling of the species of alfalfa bees with the distance from sod and bush. This may be difficult to undertake satisfactorily, although the data would be valuable in determining how close alfalfa fields should be to nesting areas and to other food supplies, in order to gauge the probable efficacy of methods used to increase the numbers of alfalfa bees.

## NESTING

### Natural Nesting Sites

As already shown in Table 4, the species of *Megachile* that trip alfalfa prefer certain habitats. This is related to the places in which they nest. The only species restricted to the sandy jackpine area is *M. latimanus*.

which nests in the ground (Mitchell, 50; Pearson, 58). *M. melanophaea* was found in both the jackpine and poplar areas but, as it also nests in the soil (Graenicher, 30), it is likely to be associated with the better-drained sandy soils of the jackpine habitat.

Three important species were closely associated with the poplar area only. These are *inermis*, *nivalis* and *relativa* and the first two have been reared at White Fox from logs. No previous nesting records are available upon *nivalis* but *inermis* has been reared from rotten apple wood by Sladen (69). *M. relativa* has been reared from a tunnel in a bank by Hicks (35) and has been recorded as possibly nesting in logs (Mitchell, 50). Guignard (34) has recorded *brevis* Say as nesting in sunflower stems, the only specimen placed as such in his collection in Ottawa proving to be *relativa*.

The remaining, and most important, species is *frigida*, which occurs commonly in both the poplar and jackpine areas. It has been recorded as nesting in a rotten log (Mitchell, 49). At White Fox many *frigida* adults were found in holes in logs but no individuals were reared, apparently because the nest-building females were ousted by females of *nivalis* and *inermis*. The numerous locality records of Mitchell (49) show the affinity of *frigida* to wooded areas, apparently including some river banks in otherwise typical prairie areas.

The records in Table 4 indicate that bumble bees, too, have certain preferences in habitat. However, these preferences are not likely to be closely related to nesting, since bumble bees normally nest either at the ground surface or below it (Sladen, 66; Plath, 60). Furthermore, as bumble bees are able to range far afield because of their powerful flight, the records for *Bombus* may reflect their preferences for certain flowering plants rather than for a type of nesting area.

From these nesting records it is evident that in northern Saskatchewan many nesting sites must have been destroyed by breaking up the raw land. Furthermore, as the farmer normally breaks the most open parts of his land first, and as these grassy and lightly timbered areas are sunny and contain all of the nesting sites, a disproportionately high number of nests and nesting sites are usually destroyed with the advent of farming.

Since the restoration of these sites is at best a gradual process, it must often be necessary to preserve the remaining areas, as well as to supplement them as far as possible. The locating of alfalfa fields near residual nesting areas and the preservation of some brush along the fences and roadsides were advocated by Salt (65), who also noted that these residual areas should be undisturbed by both farm machinery and flood waters. Possibly the most valuable method of encouraging nesting is the broadening of the narrow sunny strips that normally surround a field. Such strips could be made more attractive to bees by various methods that are discussed later.

Increases in the number of nesting sites may not necessarily result in the presence of more bees, since factors such as food may be of greater importance. Nevertheless, even if there were no scarcity of nesting sites, the establishing of new nesting areas in or near the alfalfa may still result in higher yields of alfalfa seed. The yield of seed is frequently controlled by the early fall frosts and the early tripping of the alfalfa therefore is of

considerable importance. The early tripping by bees is affected by the amount of sunshine and warm weather, by the time taken by the bees to find nesting sites, and by the time needed to fly between the nest and the alfalfa. The limitations set by the last two factors can be modified by suitable nesting areas being close to the alfalfa.

Bees may spend considerable time in looking for suitable nesting sites. A queen bumble bee may spend weeks in this search (Plath, 60) and any saving of time during the spring means the earlier production of workers in the colony and a great increase in the number of worker bees available to trip the earlier bloom. Leaf-cutter bees, however, have to search intermittently throughout the season for nesting sites so that the establishment of sufficient nests near the alfalfa would materially aid the leaf-cutter bees at this time.

Nevertheless, the locating of sufficient nesting sites near the alfalfa may not be entirely advantageous for alfalfa bees. Apart from the dangers of possible overcrowding, such as increase in parasitism and disease, the bees may have to fly great distances for other food when the alfalfa is not in bloom. Thus, the grower should not only make sufficient nesting grounds available to the bees but also see that the early food supply is close. The value of both bumble bees and leaf-cutter bees to the grower should then be considerably greater during the critical time of the early blooming of alfalfa.

#### *Artificial Nesting Sites*

The increasing of bee populations by preparing artificial nesting sites has been advocated by several authors, although no practical work has been carried out in a style that would benefit the seed grower. The spreading and rolling of gravel has been suggested as a means of providing nesting places for *Megachile perihirta* Ckll. in southern Alberta (Sladen, 68); as Sladen believed this species to nest in steep gravel banks, the gravel should not be strewn but rather piled in hollows and covered with soil on all but the sunny side. Such methods do not appear of use at White Fox. It is also of interest to note that *perihirta* is believed by Hicks (35) to nest in non-sandy soil with only a few pebbles in it.

The growing of hollow-stemmed plants may be useful in providing nest sites for *Megachile* species. Pieces of elder stem with the pith removed have been used by Balfour-Browne (3), who also found glass tubing plugged at one end to be of value. At White Fox the stems of *Heracleum lanatum* Michx. (cow parsnip) were tied in small bundles and left in sunny places; however, no bees were attracted. As noted above, sunflower stems have been selected for nesting by *relativa* (Guignard, 34). Old mullein and sunflower stems have also been used by *montivagus* (Hicks, 35), while upright stems of both sumac and burdock were found attractive to *M. centuncularis* L. (*infragilis* Cress.) by Graenicher (31). Since there is some suggestion, as shown above for *relativa*, that species of leaf-cutter bee may perhaps be found in both log tunnels and the hollow stems of plants, it may be advantageous to plant hollow-stemmed plants in the fringe around the alfalfa field. The ease with which cultivated sunflowers can be grown suggests that the old stems of this plant may be scattered advantageously along the edges of the field.



Species of *Bombus*, too, have been induced to use artificial nesting sites placed in the ground. This phase has been investigated in England by Sladen (66) and his methods have been adapted by Frison (23) and Plath (60). Great variation occurs in the suitability of species to semi-domestication in these nests (Frison, 25 to 28) and none of the species studied by Frison are known to visit alfalfa. The value of such nests in the field is problematical, depending probably upon the extent to which the colonies could flourish when neglected by man.

As bumble bees often nest under boards and duckboards, nesting may be encouraged by placing these in well-drained open spaces near the alfalfa fields. The boards would also protect the nests from excessive dampness and flooding, which Sladen (66) reported as causing great losses in bees. The boards are favourite nesting places for mice and the deserted nests are very attractive to queen bumble bees (Sladen, 66; Plath 60, Rau, 62). The placing of empty mouse nests in a natural manner under these boards would encourage such nesting; the old nests of small birds have also been found attractive in making nests (Frison, 25). However, no testing was carried out on this at White Fox.

At White Fox the nesting habits of wild bees were studied only for the alfalfa species nesting in logs and, to a very minor extent, in stems. This choice was made because these species were considered to have less chance than the ground-nesting species of finding nesting places, because this group included the valuable species *frigida* and because some success in this had already been obtained by Mr. W. D. Clarke, a local grower.

The method used by Mr. Clarke was both simple and practical. About July 30, 1943, approximately 100 holes were drilled into logs of white spruce by Mr. Clarke on his farm about 20 miles north of White Fox. Most of the logs were part of his log cabin but one of them was part of a fallen tree that was lying in a horizontal position about 2 feet above the ground. The holes in these logs were about 4 inches deep,  $\frac{1}{4}$  inch in diameter, spaced 2 to 3 inches apart and slanted upwards slightly to prevent water running into them. They were located on the south side of the logs since it was observed that leaf-cutter bees were not attracted unless the holes were exposed to a maximum amount of direct sunlight. Almost immediately, leaf-cutter bees investigated the holes and within 2 or 3 days were carrying in pieces of leaf. Within the next 2 or 3 weeks, at least half of the holes were occupied and by the end of August several had been sealed with mud plugs. A portion of fallen tree containing several sealed holes was taken to the laboratory at Saskatoon and about October 10 two adult *nivalis* bees emerged from one of the holes. In the spring of 1944 a close watch was kept on the holes in the log cabin. The first bee emerged on June 6 and emergence from several other holes continued for several days; this emergence was probably premature because of the heat from the house; however, *M. nivalis* was collected, June 13 and 15 in 1943, while visiting wild flowers. Late in the season many of these same holes were cleaned and reoccupied by bees.

During 1944 approximately 2000 holes were bored in logs and stumps at various points in the White Fox area by Mr. Clarke and the authors. White spruce, jackpine, white poplar and cedar were used. Later, bee

nests were found in the logs of all these trees. About June 29, bees were found resting in the holes during the evening and a few days later nesting activities were noted. A number of bees actively engaged in building nests in these holes were captured and *M. frigida*, *inermis* and *nivalis* later were identified from these specimens. While some of the logs were well filled with nests, less than half of the tunnels in other logs were used. The number of tunnels in a log varied from 20 to 60, depending upon the size of the log. The logs were separated by at least several hundred yards. Two of the best filled logs were placed on their sides about a foot off the ground. However, while bees search for nests at this height they can nest in artificial holes 3 and 4 feet above the ground and they frequently investigate holes in dead stubs of trees to a height of 10 to 15 feet. A log containing 80 tunnels was placed along the edge of an alfalfa field and, as nearly all these tunnels contained nests later, this may be a sound practice to ensure more uniform tripping.

The number of nests in each tunnel varied considerably. Those of *inermis* averaged 4.2 from 14 holes and showed a range of 2 to 7; those of *nivalis* averaged 3.5 from 23 holes, ranging from 1 to 6. These figures have a practical bearing upon deciding the most suitable depth for the holes; at White Fox these were about 6 inches deep and 5/16 inches in diameter. Since the nests are somewhat under 1/2 inch in length, this size appears to be long enough for these species.

In order to study the insects to be found in *Megachile* nests, parts of 5 of these logs were shipped to Ottawa in the late autumn. The logs were kept outside under snow until January, and then gradually brought up to room temperature. The logs were split to take out the nests, each of which was placed in an air-tight tin with its record of the hole from which it came and its position in the tunnel. The rearings from these nests are shown in Table 5.

TABLE 5.—REARINGS FROM BEE CELLS IN ARTIFICIAL LOG COLONIES

|                            | Log |    |    |    |   | Totals |
|----------------------------|-----|----|----|----|---|--------|
|                            | 1   | 2  | 3  | 4  | 5 |        |
| <i>Megachile inermis</i> ♂ | 4   | 6  | —  | —  | — | 10     |
| <i>Megachile inermis</i> ♀ | —   | —  | —  | —  | — | 0      |
| <i>Megachile nivalis</i> ♂ | —   | 8  | 14 | —  | — | 22     |
| <i>Megachile nivalis</i> ♀ | —   | —  | 8  | 1  | 1 | 10     |
| <i>Coelioxys</i> ♂         | —   | —  | 4  | —  | — | 4      |
| <i>Coelioxys</i> ♀         | —   | —  | 5  | —  | — | 5      |
| <i>Ichneumon</i> sp. ♂     | —   | —  | —  | 1  | 1 | 2      |
| <i>Ichneumon</i> sp. ♀     | —   | —  | —  | 4  | — | 4      |
| Chalcidoid colony          | —   | 1  | —  | 2  | — | 3      |
| <i>Anthrax</i>             | —   | 2  | —  | —  | — | 2      |
| <i>Physocephalus</i>       | —   | —  | —  | 1  | — | 1      |
| Mould                      | 1   | —  | —  | 2  | — | 3      |
| Total number of bee cells  | 5   | 17 | 31 | 11 | 2 | 66     |

The immature bees, arbitrarily defined as those in which the sex could not be externally recognized, were identified to species, with the exception of one individual, by the mandibular teeth of the larva and by the colour and texture of the cocoon. The cocoon is weak and pale yellow in *inermis* but sturdy and dull red in *nivalis*. Another guide was the identification of other nests in the same tunnel, since the nests in each appeared to be made by the same individual bee. Belief in the individual occupation of a nesting hole was supported by the adult bees emerging from each representing, with only one exception, the same sex.

The *Megachile* bees reared from these nests belonged to only two species, *nivalis* and *inermis*. The absence of *frigida* was surprising since many individuals of this species showed great interest in the tunnels. About the first of July only *frigida* was found in the logs, twelve males and eight females being taken at random for identification. Furthermore, at this time many other males were seen facing outwards at the opening of the tunnels and these were readily distinguished from *nivalis* and *inermis* by their enlarged, bright yellow protibiae. Presumably, the females of *frigida* are not as aggressive as those of *inermis* and *nivalis* and therefore allow the latter to appropriate the tunnels selected by *frigida* for nests. Possibly slightly smaller tunnels would have the effect of excluding *frigida* and *inermis*, which are somewhat larger species, should this be thought desirable.

This substitution by *inermis* would seem to be a disadvantage because this species is not only a slow tripper (Table 3) but also, according to Table 4, is not common on alfalfa, preferring sow thistle and fireweed. Although *nivalis* is a quick and efficient tripper, the few records of its flower visits (Table 4) are not sufficient to soundly establish this species as a regular visitor to alfalfa. Indeed, if the suggestion of Mitchell (51) is correct that *nivalis* may be a form of *relativa*, then *nivalis* would appear to visit other plants more frequently than alfalfa. On the other hand, there may be plenty of small natural tunnels available for *nivalis* but only a few tunnels large enough for *inermis* and *frigida*; in this case any increase in the nesting of the two latter species would result in more tripping. The immediate results of using these logs are therefore of uncertain value to the grower of alfalfa seed.

While progress has been made by using these logs, yet further study is needed before the method can be freely advocated. Consideration must be given to their attractiveness to different species being perhaps affected by the width of tunnel. The undesirable species may be excluded to a great extent by placing the logs within the fields. Possibly the tunnels should be scattered thinly around or in the field to reduce parasitism, to reduce the flying-time of individual bees and to reduce irregularities of seed-setting within the field. The present methods can therefore be considered as practical but needing modifications in order to conform more closely with the biological needs and habits of this group of alfalfa bees.

#### FORAGING

The supplies of food available to the alfalfa bees must of course be considered in any program for increasing or maintaining bee populations. Under natural conditions in the nearctic area there is a large assortment of bloom in both spring and fall, while the supply in late summer is scanty.



The advent of farming at White Fox has greatly reduced the early and late blooms, with the exception of dandelion which has now invaded practically all roadsides and older alfalfa fields and has both early and late flowering periods. Summer bloom has been increased many times by the introduction of farm crops and weeds such as alfalfa, clovers and sow thistle. The grower of alfalfa seed thus needs to know (1) how much the alfalfa bees are affected by the losses in native flowers and (2) to what extent these bees visit other plants while the alfalfa is flowering. The answers are not simple, since bumble bees and leaf-cutter bees have quite different life cycles, and since the species within these two groups vary in their flower preferences. The two major groups are therefore discussed separately.

During the summer months the alfalfa bees usually have an abundance of food supplied by cultivated crops and by weeds. The bees must then be restricted to alfalfa bloom as far as is reasonably possible. According to Salt (65) the blossoming of the alfalfa-seed crop should be accompanied by the cutting of sweet clover, white Dutch clover and alfalfa hay fields. Red clover grown for seed in plots is also attractive to *Bombus* to some extent although only slightly so to *ternarius*, *terricola* and *rufocinctus* (Morrison, 54). At White Fox among the most prominent flowering plants are dandelion, sow thistle and fireweed. Dandelion is abundant in the alfalfa fields and apparently at present ineradicable. Indeed, from the standpoint of bee populations, eradication of dandelion may be inadvisable since it does not compete strongly with alfalfa in the summer season and may be a useful source of food in the spring and fall which are its normal periods of bloom. Sow thistle may be abundant throughout fields but usually is to be found along adjacent roadsides and nearby waste places. It can be checked by cultural control or mowing. Fireweed occurs chiefly in open spaces after brush-burning and may cover extensive areas. Control by mowing is practical where sticks and stones do not interfere. Otherwise chemical sprays would have to be used if adequate control is to be secured. The effects of these plants upon the flower visiting of various species of alfalfa bees are discussed later.

The amount of food available for alfalfa bees may be locally affected during times of scarcity by the feeding of other wild bees and also of honey bees. As noted previously, the numbers of the latter make the keeping of honey bees on a large scale a possible threat to the growing of alfalfa.

The visits of alfalfa bees to other flowers could be recorded only by carrying out general collections upon selected species of plants and this unfortunately had to be restricted to the time of alfalfa bloom in 1944. Information is therefore needed upon (1) the visits made to plants previous to and later than this time and (2) upon the activity of the bees in gathering pollen or nectar or both. Additional data could be obtained from captured bees by analysing their pollen.

Although no faunal list of *Bombus* has been compiled for Saskatchewan, that of Neave (55) for Manitoba can be adapted to the White Fox area. Records for *Megachile* (subgenera *Anthemois*, *Delomegachile* and *Xanthosarus*) in Saskatchewan are included in Mitchell's monograph of the genus (49, 50, 51).

Records of the flower visits of *Bombus* have been published by Lovell (41), Lutz and Cockerell (42) and Plath (60), while Mitchell (49, 50, 51) has reviewed those for the species of alfalfa bees in the genus *Megachile* found at White Fox. These records are indicative mainly of the possible attractiveness of these flowers at White Fox, because the records are not quantitative, and because the distractive flora at the place of record is not similar to that at White Fox. Nevertheless, records of such visits assume greater importance during periods when pollen or nectar is scarce. The above lists of records for these species suggest that they are capable of feeding from a wide range of plants.

*Foraging by Bombus spp.*

At White Fox the important species of *Bombus* during 1944 were *terricola*, *ternarius* and *rufocinctus* (Table 4). According to Plath (60), the first two species appear early in the spring and, according to Neave (55), *ternarius* emerges later than *terricola*. A comparison of dates for specimens taken at Ottawa suggests that *rufocinctus* is as early as *terricola* and earlier than *ternarius*. At this time only a small amount of bloom is needed for the population and, as the records of flower visits show that the three species feed from a wide range of flowers, this food could be assured by the reservation of small scattered areas of suitable ground. Such reservations could easily be planted with cuttings of willows and dogwoods, if these plants are not present. The finding of a suitable nesting site is the first task of the queen and this may take as long as 6 weeks (Plath, 60) but, once this is done, brood appears in about 22 days from the time that the eggs are laid (Sladen, 66; Plath, 60). To feed the larvae, the queen brings both pollen and nectar into the nest and, once workers appear, increasing quantities of both foods are needed. The sources of food at this time are presumably native flowers until the sow thistle and alfalfa begin to bloom. As shown in Table 4, dandelion was rarely visited by bumble bees during the alfalfa bloom. These increasing demands for pollen and nectar do not seem to be met by any of the farm plants until the alfalfa bloom. Dandelion begins to flower before alfalfa but, as there are no records of such visits by *Bombus* spp. (41, 42, 60), and as only two visits to dandelion by bumble bees are noted in Table 4, this plant may be unimportant. Since no records were available at White Fox before the alfalfa bloom, dandelion may still be merely less attractive to these bumble bees than alfalfa.

During the alfalfa bloom the alfalfa bees in the genus *Bombus* showed specific preferences towards various farm weeds and native plants. As shown in Table 4, *B. terricola*, the most important species, is only distracted to sow thistle to a minor extent and scarcely at all to dandelion and fireweed. On the other hand, sow thistle is distractive to a serious extent to both *ternarius* and *rufocinctus*. The fireweed clumps used for records were not close to the alfalfa so that competition between the two plant species was not as keen. Nevertheless, fireweed may be distractive to *ternarius* to a serious extent, although this plant flowers somewhat later than alfalfa and therefore may not be important during the first 2 or 3 weeks of alfalfa bloom when most of the seed to be harvested is set. Thus, of the three plants studied, sow thistle seriously distracts alfalfa bees, fireweed is probably of minor importance and dandelion negligible.

The emergence of new queens and males from the nest is accompanied by the disintegration of the colony, the queens mating and seeking hibernation quarters soon afterwards; consequently the number of flower visits by bumble bees diminishes rapidly. The time of emergence of the queens and males varies greatly, this depending both upon the species and weather (Plath, 60). At White Fox a survey of bumble bees was made about August 1, 1944, but the only males taken were 2 of *terricola* on August 1 and 3; in contrast to this a specimen of *terricola* was taken at Crean Lake, Prince Albert National Park, on September 11 by Cockerell (12). As *terricola* has been noted as having early males (Plath, 60), the disintegration of the colonies may not be general for even *terricola* until later. *B. ternarius* is recorded in New England as having males in August and September, a month after *terricola* (Plath, 60) so one may expect *ternarius* to be later at White Fox, too. Records taken at Ottawa for *terricola*, *ternarius* and *rufocinctus* suggest that the two latter species have males at about the same time.

From these data it is evident that the food requirements of *Bombus* for August and September fluctuate greatly. After about August 15 the alfalfa bloom rapidly disappears and the bees are largely dependent on native flowers. A severe frost in early August is likely to cause the weaker colonies to die out, since, according to Plath (60), food at this time is scarce. Among the blooms visited in the fall by *terricola* and *ternarius* are *Solidago bicolor* L. (Lovell, 40), while these species also were found on goldenrod at White Fox (Table 4). As *Solidago* species often produce much pollen and nectar, even during cool weather (Lovell, 41), beds of these plants should be preserved.

#### *Foraging by Megachile spp.*

The feeding habits of *Megachile* differ greatly from those of *Bombus*, since they are solitary bees. Their larvae are not fed in a colony but, instead, the individual nest for each egg is provisioned with enough food to last the young bee until maturity. This habit protects the immature bee against starvation caused by an unseasonable decrease in the amount of suitable bloom, an event that may exterminate whole colonies of bumble bees.

The duration of the nesting time is dependent upon the amount of warm weather, since the adults fly only on warm sunny days. According to the record dates of leaf-cutter bees in the Canadian National Museum, adults in the Canadian Zone fly from about the middle of June until the middle or end of August. However, the length of this period of course is affected by variations in the weather from one season to another.

The shortness of the adult stage in *Megachile* allows the adults of some species to utilize only a few species of plants. Alfalfa bees in the genus *Megachile* may have a tendency towards this condition because (1) alfalfa supplies nectar profusely during warm weather (Lovell, 41), its pollen also being abundant and (2) alfalfa has a long period of bloom unless an unseasonable frost occurs.

Before the blooming of the alfalfa there is a varied and abundant bloom of native plants and dandelion. Alfalfa bees in this area belonging to the genus *Megachile* should be able to find sufficient food at one plant



species or another to prevent actual starvation in any typical area in which the native bloom has been reasonably conserved. Even if the actual nesting at this time is retarded for lack of bloom nearby, this should not have much effect upon the number of bees for the following year.

About the time that the alfalfa begins to bloom, the alfalfa fields have an abundant bloom of dandelion, a plant that produces both pollen and nectar abundantly in warm weather (Lovell, 41). Dandelion was not recorded at White Fox as being visited by either *M. frigida* or *latimanus* and the visits of other, less numerous, tripping species were few (Tables 3, 4). Nevertheless, *latimanus* and *inermis* have been recorded from this plant by Mitchell (49). Dandelion may therefore be merely less attractive to these alfalfa bees than alfalfa and, if so, may play an important role in providing food during the critical feeding period before the alfalfa blooms.

The bloom of two other common plants, perennial sow thistle and fireweed, have been noted by Knowles (37) to attract *Megachile* species in alfalfa fields at White Fox. In Table 4 it is shown that, while *frigida* was rarely attracted to sow thistle, yet *latimanus*, *inermis* and *relativa* were visitors to an appreciable degree. Fireweed was quite attractive to most of the bees, although it was not close to the alfalfa and therefore not strictly in competition. While the control of both of these plants may be desirable from the viewpoint of tripping, yet these plants may be beneficial in supplying food after the first fall frost has killed the alfalfa bloom.

As cooler weather sets in during the fall, there is a diminution or cessation of nest-building by *Megachile*. An abnormally early fall frost would kill the alfalfa bloom and, if the bees survived, would make them dependent upon the native bloom, which is scarce at this time. However, available collection dates suggest that these heat-loving bees are killed or inactivated by a fall frost.

A comparison of the feeding habits of *Megachile* and *Bombus* shows that the population of the latter alone is prone to early spring starvation, while early fall frosts are also likely to reduce the population for the next year much less severely in *Megachile* than in *Bombus*.

#### DISEASE AND MOULD

No investigations were made upon these factors. Nests in both soil and logs have been found filled with mould and this was true also of many nests built in the drilled holes of an incompletely dried log of poplar. Mould also was found in 3 nests of sound wood (Table 5). No conclusion can be reached upon these moulds being saprophytic or parasitic, although, if saprophytic, as seems likely, the use of sound wood seems to be a logical means of reducing losses of bees in artificial nesting sites.

#### PARASITES AND PREDATORS

Changes in the habitat and environment of an insect group are usually followed by fluctuations in the populations, not only within this primary group, but also within a secondary group formed by their parasites and predators. The densities of these two groups, their specific compositions and their inter-relationships should be of special interest in the case of the

alfalfa bees at White Fox, since these bees have been subjected to abnormally great environmental changes caused by the destruction of much of the vegetation.

The parasites and predators of bumble bees, and the inquilines of their nests, have been studied by Frison (24) in Illinois. The conopids, or large-headed flies, commonly parasitize adult bumble bees (Meijere, 45; Frison, 24) and are likely to be overlooked as a factor that reduces the efficiency of adults. Their predators include mammals, such as mice and moles (Frison, 1.c.), and asilids (Bromley, 7). Frison's extensive list of inquilines has been supplemented with some lepidopterous records by Milum (47). On the other hand, although bumble bees have a long list of enemies, it is worth recording that losses in bumble bees can be slight. A nest of *B. americanorum* F. was dug up late in the season by Rau (62) and contained 138 adults and 238 immature bees; only 6 workers had been lost from the nest and there was no evidence of the presence of social or other parasites. Nesting records, taken in a similar manner at White Fox, would assist greatly in making population studies.

Adult bees of both *Bombus* and *Megachile* at White Fox were frequently carrying many hypopi of various acarid (tyroglyphid) mites. As these are merely in the dispersal stage, they do not normally inhabit the nests and, in any case, since their mouth parts are vestigial, they cannot attack either the immature or adult bee. These mites are therefore of no direct importance as parasites or predators (Michael, 46).

At White Fox both the probable crowding of alfalfa bees into restricted nesting areas and the establishment of artificial colonies in logs may favour the increase of parasites and predators. Studies upon nesting populations were confined to those nests that were built in the log colonies, rather than those in natural nests. This was done, in part, to save time in searching for nests and in handling but also, in part, to establish the identities of the colonizing bees.

The percentage of harmful inquilines in nests of artificial colonies may be affected by the size of the colony. Although a female inquiline may find nests more easily, particularly so if she herself matured in the colony, yet her use of such a nest may result in a higher loss of her progeny through other noxious inquilines that also found the nests more easily. Again, the inquiline newly emerged from a colony may lose the location of the nest by satisfying her needs such as feeding from flowers or finding a mate. The grouping of the nests may also mean that the numbers of the bees around the nests may deter inquilines from entering. The effect of the size of colony upon the inquiline population is therefore not necessarily beneficial to either bee or inquiline.

At White Fox, data were obtained upon the numbers of inquilines found in new colonies but not in any established for longer than a year. The reported proportions of inquilines may therefore be lower than if older colonies had been examined. The results, shown in Table 5, should be considered only as a preliminary study to a problem that requires intensive work for some seasons. These figures, however, suggest that colonization has not been of great advantage to the inquilines and that any increased ability to find bee nests, if present, has been neutralized by one or more factors.

Among these factors may be the deterrent effect of the continued activity to be found about a large vigorous colony during flying weather. This is in turn affected by the distance of the colony from the sources of pollen, nectar and such leaves as are suitable for building nests. At White Fox an artificial colony of *M. inermis* and *nivalis* was well established in a cedar log within 10 yards of both alfalfa and poplar (Table 5, log 2). This attracted numbers of bees of the species *Coelioxys moesta* and *C. lucrosa*, the larvae of these being predators within the cells of *Megachile* (Graenicher, 30, 31). Nevertheless, no trace of *Coelioxys* was found when the nests were dissected after rearing was completed. On the other hand, another log (Table 5, log 3) with nests of *M. nivalis* had *Coelioxys* in 5 out of 18 occupied tunnels. The numbers of *Megachile* bees in and about Log 2 may have been sufficient to ward off the *Coelioxys* attacks.

It is worth recording, at this point, that Log 2 was also attractive to cuckoo wasps (*Chrysis* sp., probably *coerulans* F.). Although no individuals of either *Chrysis* or *Coelioxys* were seen to enter the log, yet the apparent interest of the wasps suggested strongly that they also intended to use the nests. No specimens of these wasps were reared nor was other evidence seen of their being inquiline. Records from two of the monographs on the family (Mocsary, 52; Bischoff, 6) suggest that these chrysidids may have been searching for nests of another hymenopterous group, possibly *Osmia*. However, Friese (21) recorded the chrysidid genera *Holopyga* and *Hedychrum* as being reared from *Megachile*, while Dalla Torre (13) had seven palaearctic records of such chrysidid parasitism, including four for *Chrysis*. The ability of *Chrysis* to oviposit in the solitary, and the colonial, nests of *Megachile* may be worth studying.

Records of inquilines in the nests of leaf-cutter bees are scattered in the literature. Those in the works of Bezzi (5), Dalla Torre (13-15), Friese (21, 22), Gahan (29), Malloch (43), Morley (53) and Thompson (71) are listed in Table 6. Additional nearctic records were also added. Most of the records are European but it is interesting to note that the inquilines in the palaearctic and nearctic regions so frequently show similarity by occurring together in certain, widely separated genera or groups; indeed, as *M. centuncularis* is now considered as holarctic (Mitchell, 49), one may suspect that some of the inquiline species may also be holarctic.

At White Fox 156 nests were taken from artificial colonies. Unfavourable temperatures during shipping apparently caused many deaths among the larvae, so that only 63 nests produced either bees or inquilines. These are recorded in Table 5. The associations of the bee species with their inquilines are as follows:—

*Megachile nivalis*: *Coelioxys moesta* Cress., *Ichneumon* sp., *Anthrax irrogata* Say, *Physocephalus dakotensis* V.D.

*Megachile inermis*: *Ichneumon* sp., *Dibrachys* sp.

Important amongst the harmful inquilines in *Megachile* nests are species of the megachilid genus *Coelioxys*, in which the newly hatched larva kills the *Megachile* larva and then feeds upon the pollen and nectar in the nest (Graenicher, 32). *M. latimanus* and *M. melanophaea* var. *wooteni* Ckll. are both preyed upon by *C. dubitata*, while *M. frigida* (*vidua*) is



TABLE 6.—HOLARCTIC PARASITES AND PREDATORS IN *Megachile* NESTS<sup>1</sup>

| Order       | Family        | Genus                 | Nearctic species                            |
|-------------|---------------|-----------------------|---|
| Coleoptera  | Meloidae      | <i>Meloë</i>          | —   |
|             | Cleridae      | <i>Trichodes</i>      | —   |
| Diptera     | Bombyliidae   | <i>Anthrax</i>        | <i>irrogata</i> Say (new record)            |
|             |               | <i>Spogostylum</i>    | —   |
|             | Conopidae     | <i>Physocephalus</i>  | <i>dakotensis</i> V.D. (new record)         |
|             | Sarcophagidae | <i>Miltogramma</i>    | —   |
| Hymenoptera | Ichneumonidae | <i>Spilocryptus</i>   | sp. (33)                                    |
|             |               | <i>Kaltenbachia</i>   | —   |
|             |               | * <i>Ichneumon</i>    | sp. (new record)                            |
|             | Leucospidae   | <i>Leucospis</i>      | <i>affinis</i> Say (15)                     |
|             | Torymidae     | <i>Monodontomerus</i> | ? <i>emarginatus</i> Gahan (29)             |
|             | Pteromalidae  | <i>Dibrachys</i>      | sp. (new record)                            |
|             |               | * <i>Semiotellus</i>  | <i>cupraeus</i> Prov. (34)                  |
|             | Eupelmidae    | <i>Calosota</i>       | —   |
|             | Eulophidae    | <i>Melittobia</i>     | <i>megachilis</i> Pack. (57)                |
|             | Mymaridae     | * <i>Pteratomus</i>   | <i>putnamii</i> Pack. (57) (Hyper-parasite) |
|             | Chrysididae   | <i>Chrysis</i>        | —   |
|             |               | <i>Stilbum</i>        | —   |
|             |               | <i>Hedychrum</i>      | —   |
|             |               | <i>Holopyga</i>       | —   |
|             | Sapygidae     | <i>Sapyga</i>         | <i>fulvicornis</i> Cress. (39)              |
|             | Megachilidae  | <i>Stelis</i>         | —   |
|             |               | <i>Dioxys</i>         | <i>coloradensis</i> Cress. (35)             |
|             |               | <i>Coelioxys</i>      | <i>dubitata</i> Sm. (32, 35, 63)            |
|             |               |                       | <i>grindeliae</i> Ckll. (35)                |
|             |               |                       | <i>hicksi</i> Ckll. (11)                    |
|             |               |                       | <i>lucrosa</i> Cress. (32)                  |
|             |               |                       | <i>modesta</i> Sm. (31, 64)                 |
|             |               |                       | <i>moesta</i> Cress. (new record)           |
|             |               |                       | <i>octodeniata</i> Say (20, 35)             |
|             |               |                       | <i>ribis</i> Ckll. (31)                     |
|             |               |                       | <i>rudis</i> Ckll. (11)                     |
|             |               |                       | <i>sayi</i> Rob. (64)                       |

<sup>1</sup> Compiled from the literature, and from the records of the authors for the White Fox area.

\* Not recorded from the palaearctic region.

similarly attacked by *C. lucrosa* (Graenicher, 32). These two species may therefore be important factors in keeping down the population of the alfalfa bees in the genus *Megachile*. Four males and 5 females of *Coelioxys* were reared, all from the same colony (Table 5, log 3). The females run readily to *moesta* in the key by Sladen (67) and are believed to be that species. The associated males also run by this key to *moesta* except in their having small round foveae on the second tergite. The association of the forms seems as sound as one can obtain without rearing both sexes from the same mother; the failure to obtain both sexes from the same nest is of course not especially significant. The male of *moesta* has only been described as such by Sladen (*l.c.*) and therefore the correctness of his associations has not been confirmed by other workers.

Only one species of ichneumonid was reared from the nests. This belonged to the genus *Ichneumon* (*s.s.*), both males and females being reared. This species may prove to be *I. brevis* Morley but the type of this is inaccessible; otherwise the species appears to be new. The female has a long ovipositor for piercing both the individual nests and the mud

cap that seals each nest entrance. In one case 3 females were reared from the second to fourth nests in the same hole. One may judge from this and from the usual number of nests in a hole that most of the leaf-cutter nests are liable to parasitization by this species after the nest hole has been completely filled by nests.

A relatively large number of chalcids have been recorded in Table 6 as parasites of leaf-cutter bees. The potential parasitism by chalcid species is suggested by the records of Guignard (34) and Putnam (61). Guignard found every larva in a colony of 20 nests of "*M. optiva*" (apparently *melanophaea*) were parasitized by *Semiotellus cupraeus* Prov. Putnam recorded that a single nest of *M. centuncularis* contained over 150 adults of *Melittobia megachilis* (Pack.) and, according to Packard (41), these were in turn parasitized by *Pteratomus putnamii* Pack.

At White Fox the only adult chalcids reared from *Megachile* were 2 males and 23 females of *Dibrachys* reared from a single nest of *M. inermis*. Two chalcid colonies from another site contained 26 and 32 unidentified larvae. In none of these was the nest pierced by the chalcid mother.

Both the reduction in the area of possible nesting sites and the use of artificial colonies in logs are factors that tend to assist chalcids in their search for hosts, because of their relatively weak flight. Indeed, this colonization seems ideal for *Melittobia*, a genus in which the males are recorded as wingless and the females as fully winged but virtually flightless (Browne, 8; Buckell, 9).

The means by which large numbers of adult chalcids can develop from one small bee larva are of interest. The parasitizing of the egg or of the earlier larval stages would seem to tax too severely the ability of the young bee to supply food to the numerous parasitic larvae. On the other hand, later parasitizing may seem unlikely because (1) the finished nest is usually inaccessible to the chalcid and (2) an excessively long period would elapse before the bee larva would be capable of feeding the chalcid larvae and, during this time, either the mother would have to survive or her eggs lay dormant in the nest.

A clue is suggested by the life cycle of the European chalcid, *Melittobia acasta* (Wlk.), which parasitizes leaf-cutter bees, other wild bees and wasps. The following observations of Browne (8) on this species are pertinent. The adults of *M. acasta* are often found enclosed in bee cells, are able to feed on solitary young larvae without hindering the host development, and can remain as long as 50 or 60 days beside a future host before ovipositing in it. These qualities together would seem to allow this chalcid to delay oviposition until the host is sufficiently large to nourish the parasitic larvae. The bee-food stored in the nest may also be of value in lengthening the life of the mother chalcid. The feeding of adult female chalcids upon future hosts is found among other chalcids, including the nearctic species *Melittobia chalybii* Ashm. (Buckell, 9), the holarctic *Dibrachys cavus* (Wlk.) (Faure and Zolstarewsky, 19) and a number of species in various chalcidoid families (Clausen, 10). This feeding habit may therefore be used to synchronize the life cycle of *Dibrachys* and other chalcid parasites with that of its bee host.

On the other hand, there is evidence that suggests that more than one generation of parasites may be produced in a nest. According to Howard and Fiske (36), only the females of *M. acasta* are long-lived. Such females lay only 4 or 5 eggs on individual larvae of the gipsy moth, *Porthetria dispar* L. These eggs produce only males, mating then takes place with the mother and the resulting eggs produce female parasites. At White Fox the parasitized nest contained 2 males and 23 females, the males and some females being dead when first examined. These numbers are suggestive of the conditions reported by Howard and Fiske (*l.c.*) but unfortunately there is no proof that the males did not enter the nest while it was being built. Detailed observations upon the life cycle of this species of *Dibrachys* at White Fox may show the method of synchronization.

The flies reared from the nests of leaf-cutter bees at White Fox belonged to two species, *Anthrax irrogata* Say<sup>1</sup> in the Bombyliidae (bee-flies) and *Physocephalus dakotensis* V.D. in the Conopidae (large-headed flies).

Although the Bombyliidae, as a family, have exceedingly varied hosts, yet their species are confined within relatively narrow limits; the genera *Anthrax*, *Argyramoeba* and *Spogostylum* are recorded by Bezzi (5) asinquilines of *Megachile* nests. There appear to be no *Megachile* records for the nearctic area, although *A. irrogata* (as *Argyramoeba oedipus* F.) has been noted as an external parasite of solitary wasps belonging to the genus *Odynerus* by Baker (4). The 2 specimens reared at White Fox were found in the second and third cells out of a row of four.

The rearing of one adult of the conopid, *P. dakotensis*, is of unusual interest. According to Clausen (10), the members of this family usually parasitize adult hosts, although there is some evidence that larvae of *Vespa* may be attacked. In the case of the White Fox specimen, there can be no doubt that the entrance of the parasite to the nest was made before the nest was complete, since the nest was intact during the winter, the nest itself being isolated in a tin until the emergence of the adult bee host and again isolated in a Schmitt box with its pinned host and some other dead bees. The conopid pupated within the abdomen of its host, as is normal for conopids. Possibly the parasite egg was laid in the normal manner upon the mother bee and was carried as a larve into the nest. However, as the eggs of this group show no embryonic development at the time of deposition (Clausen, 19), it therefore seems likely that the adult conopid laid the egg either on or near the egg of its future host and that the larva fed upon the bee-food until the time when it parasitized the bee.

From the above discussion upon the hymenopterous and dipterous parasites of alfalfa bees, it is evident that these parasites were not of major importance in limiting the number of alfalfa bees at White Fox during 1944. Nevertheless, during other seasons the importance of their role may be greatly increased or lessened; and, as already emphasized, this may be especially true if artificial nesting sites are used to increase the numbers of bees in the fields of alfalfa.

<sup>1</sup> Determined by the key published by Maughan (44).



## SUMMARY

In the White Fox district, as in others, an adequate setting of alfalfa seed is dependent upon the presence of sufficient numbers of bees to fertilize the alfalfa flowers by tripping and cross-pollinating.

At White Fox the important bees for tripping are leaf-cutter bees (*Megachile* spp.) and bumble bees (*Bombus* spp.). Honey bees are plentiful but unimportant except perhaps for their competition with wild bees for pollen and nectar during times of scarcity.

The burning of the brush and the ploughing of the sod by farmers have caused the destruction of many bee nests containing immature and adult bees, as well as spoiling many sites suitable for future nesting. Furthermore, this destruction has deprived the bees of many sources of nectar and pollen.

In the poplar areas *Megachile frigida* was by far the most important leaf-cutter bee for tripping during 1944. Other species were also useful, including *relativa*, *nivalis*, *inermis*, and *melanophaea*. In the sandy, jackpine areas *M. frigida* was still the most common species although *latimanus* was important also. The most valuable bumble bee was *B. terricola*, although *B. ternarius* and *B. rufocinctus* (s.l.) were also useful; these were found in both poplar and jackpine areas.

Suggestions are made for the restoration of nesting sites for *Bombus* and *Megachile* by a variety of ways. *Megachile* bees show strong specific preferences towards certain types of nesting sites. Bumble bees nest either in or on the earth.

The preservation of existing nesting sites and the establishment of new ones are essential for high yields of seed from well established alfalfa areas. Early nesting of bumble bees may be encouraged by placing boards 1 inch above the sod in suitable places. Artificial domiciles for bumble bees, such as those devised by Sladen and Frison, cannot be considered as practical unless supervision during the growing season can be eliminated. The alfalfa fields should be surrounded by a wide strip of uncultivated land for nesting; such areas should not be subject to flooding, to disturbance by farm machinery or to shading by heavy bush. Fence-rows and road allowances may provide nesting sites. Artificial nests, made by boring holes in logs at White Fox, have been used by *M. nivalis* and *inermis*; although the important species *frigida* has taken great interest in such nests, no specimens have been reared. The rearing of leaf-cutter bees in such colonies may affect the influence of the harmful inquilines, although such does not at present seem true. Four species of inquilines were reared.

The populations of *Bombus* spp. are probably subject to much greater fluctuations than those of *Megachile* spp. because of starvation.

Early spring bloom is needed for the *Bombus* queens emerging from hibernation. An increasing supply of bloom is then needed until the alfalfa blooms, or else the colonies will be weak during the important early blossoming of this plant. The growth of early flowering plants should be encouraged at the edges of alfalfa fields.

*Megachile* adults emerge about the middle of June. The flowers of dandelion and native plants probably provide sufficient food until the alfalfa blooms.

During the alfalfa bloom there is abundant food supplied also by other plants and the alfalfa bees must be restricted to alfalfa bloom as far as reasonably possible. The cutting of hay crops of flowering plants such as sweet clover and alfalfa should therefore be carried out before the seed crop blooms. The bloom of dandelion, sow thistle and fireweed is abundant at White Fox; dandelion is not a competitor and may be useful in supplying food before the alfalfa blooms; sow thistle is moderately attractive to alfalfa bees; fireweed is quite attractive to *M. frigida*, the most important species. Control of sow thistle and fireweed by any practical means is desirable.

An early frost in the fall may deprive the bumble bees and leaf-cutter bees unusually soon of their normally plentiful supplies of food from the alfalfa. Unless there are other suitable sources of food, such as golden-rods and sow thistle, the deaths among *Bombus* may be so numerous as to seriously lower the number of colonies in the next season. Such a famine would not affect the population of *Megachile* as greatly during the next year, since the young have been provisioned.

Field data are needed upon the flower visiting of various species in *Bombus* and *Megachile* during spring and August to ensure to these bees an adequate supply of food.

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#### REFERENCES

1. AICHER, L. C. The production of alfalfa seed in southern Idaho. Idaho Expl. Sta. Bull., 101. 1917.
2. ARMSTRONG, J. M., and W. J. WHITE. Factors influencing seed-setting in alfalfa. Jour. Agr. Sci. 25 : 161-179. 1935.
3. BALFOUR-BROWNE, F. Concerning the habits of insects. Especially bees, wasps, dragonflies and water beetles. Cambridge Univ. Press, 1925. *Abstract in* Peterson, A. A manual of entomological equipment and methods 2 : 13, St. Louis, Mo. 1937.
4. BAKER, C. F. Biological notes on some Colorado Diptera. Ent. News 6 : 173-174. 1895.
5. BEZZI, M. The Bombyliidae of the Ethiopian region. Brit. Mus. 1924.
6. BISCHOFF, H. Hymenoptera; fam. Chrysididae. P. Wytzman's Genera Insectorum, fasc. 151. Brussels. 1913.

<sup>1</sup> Recently transferred to the Entomological Laboratory, Victoria, B.C.



7. BROMLEY, S. W. Asilids feeding on bumble bees in New England. *Psyche* 43 : 14. 1936.
8. BROWNE, F. B. On the life-history of *Melittobia acasta*, Walker; a chalcid parasite of bees and wasps. *Parasitology* 14 : 349-369. 1922.
9. BUCKELL, E. R. Notes on the life-history and habits of *Melittobia chalybii* Ashmead. (Chalcidoidea : Elachertidae). *Pan-Pacific Ent.* 5 : 14-22. 1928.
10. CLAUSEN, C. P. *Entomophagous insects*. McGraw-Hill, N.Y. and London. 1940.
11. COCKERELL, T. D. A. New and little-known western bees. *Amer. Mus. Nov.* 732. 1934.
12. COCKERELL, T. D. A. The entomology of Prince Albert National Park, Saskatchewan. *Can. Ent.* 68 : 85-86. 1936.
13. DALLA TORRE, C. G. DE. *Catalogus Hymenopterorum*. Vol. VI. Chrysididae (Tubulifera). Engelmann, Leipzig. 1892.
14. DALLA TORRE, C. G. DE. *Catalogus Hymenopterorum*. Vol. X. Apidae (Anthophilidae). Engelmann, Leipzig. 1896.
15. DALLA TORRE, C. G. DE. *Catalogus Hymenopterorum*. Vol. V. Chalcididae et Proctotrupidae. Engelmann, Leipzig. 1898.
16. DALLA TORRE, C. G. DE. *Catalogus Hymenopterorum*. Vol. III. Trigonalidae, Megalyridae, Stephanidae, Ichneumonidae, Agriotypidae, Evaniidae, Pelecinidae. Engelmann, Leipzig. 1901-1902.
17. DWYER, R. E. P., and S. L. ALLMAN. Honey bees in relation to lucerne seed-setting. *Agr. Gaz. New South Wales.* 44 : 363-371. 1933.
18. ENGELBERT, V. A study of various factors influencing seed production in alfalfa (*Medicago sativa*). *Sci. Agr.* 12 : 593-603. 1932.
19. FAURE, J.-C. and B. ZOLSTAREWSKY. Contribution a l'étude biologique de *Dibrachys boucheanus* Ratz. *Rev. Path. Végét. d'Ent. Agric.* 12 : 144-161. 1925.
20. FOX, W. J. *Coelioxys 8-dentata* and its host. *Ent. News* 11 : 553. 1900.
21. FRIESE, H. Die Bienen Europa's (Apidae europeae). V. Solitare Apiden : Genus *Lithurgus*, Genus *Megachile* (*Chalicodoma*). Lampe, Innsbruck. 1899.
22. FRIESE, H. *Das Tierreiche*. Apidae I. Megachilinae. Friedländer, Berlin. 1911.
23. FRISON, T. H. Experiments in attracting queen bumble bees to artificial domiciles. *Jour. Econ. Ent.* 19 : 149-155. 1926.
24. FRISON, T. H. A contribution to the knowledge of the interrelations of the bumble bees of Illinois with their animate environment. *Ann. Ent. Soc. Amer.* 19 : 203-236. 1926.
25. FRISON, T. H. Experiments in rearing colonies of bumble bees (Bremidae) in artificial nests. *Biol. Bull.* 52 : 51-67. 1927.
26. FRISON, T. H. A contribution to the knowledge of the life history of *Bremus bimaculatus* (Cresson) (Hym.). *Ent. Amer.* 8 : 159-214. 1928.
27. FRISON, T. H. A contribution to the knowledge of the bionomics of *Bremus impatiens* (Cresson) (Hym.). *Bull. Brooklyn Ent. Soc.* 24 : 261-285. 1929.
28. FRISON, T. H. A contribution to the knowledge of the bionomics of *Bremus americanorum* (Fabr.) (Hym.). *Ann. Ent. Soc. Amer.* 23 : 644-665. 1930.
29. GAHAN, A. B. A revision of the chalcid-flies of the genus *Monodontomerus* in the United States National Museum. *Proc. U.S. Natl. Mus.* 90 : 461-482. 1941.
30. GRAENICHER, S. Some observations on the life history and habits of parasitic bees. *Bull. Wisc. Nat. Hist. Soc.* 3 : 153-167. 1905.
31. GRAENICHER, S. On the biology of the parasitic bees of the genus *Coelioxys*. (Hym., Megachilidae). *Ent. News* 38 : 231-235, 273-276. 1927.
32. GRAENICHER, S. Bee fauna and vegetation of Wisconsin. *Ann. Ent. Soc. Amer.* 28 : 285-310. 1935.
33. GRAY, H. E. Observations on tripping of alfalfa blossoms. *Can. Ent.* 57 : 235-237. 1925.
34. GUIGNARD, J. A. Beginning an acquaintance with wild bees. *Ent. Soc. Ont. Ann. Rpt.* 17 : 51-53. 1887.



35. HICKS, C. H. Nesting habits and parasites of certain bees of Boulder County, Colorado. Univ. Colo. Stud. 15 : 217-252. 1926.
36. HOWARD, L. O., and W. F. FISKE. The importation into the United States of the parasites of the gipsy moth and the brown-tail moth. U.S. Dept. Agric., Bur. Ent., Bull. 91. 1911.
37. KNOWLES, R. P. The role of insects, weather conditions, and plant character in seed-setting in alfalfa. Sci. Agr. 24 : 29-50. 1943.
38. LEJEUNE, A. J., and P. J. OLSON. Seed-setting in alfalfa. Sci. Agr. 20 : 570-573. 1940.
39. LINSLEY, E. G. Host relationships of some Sapygid wasps. (Hymenoptera, Sapygidae). Bull. Brooklyn Ent. Soc. 39 : 54-55. 1944.
40. LOVELL, J. H. The bumble bees of southern Maine. Ent. News 18 : 195-200. 1907.
41. LOVELL, J. H. Honey plants of North America, north of Mexico. Root, Medina, Ohio. 1926.
42. LUTZ, F. E. and T. D. A. COCKERELL. Article XV. Notes on the distribution and bibliography of North American bees of the families Apidae, Meliponidae, Bombidae, Euglossidae and Anthophoridae. Bull. Amer. Mus. Nat. Hist. 42 : 491-641. 1920.
43. MALLOCH, J. R. A preliminary classification of Diptera exclusive of Pupipara, based upon larval and pupal characters, with keys to imagines in certain families Part I. Bull. Ill., St. Lab. Nat. Hist. 12 : 161-407. 1917.
44. MAUGHAN, L. A systematic and morphological study of Utah Bombyliidae, with notes on species from intermountain states. Jour. Kans. Ent. Soc. 8 : 27-80. 1935.
45. MEIJERE, J. C. H. DE. Neue Beiträge zur Kenntnis der Conopiden. Tidschr. Ent. 55 : 184-207. 1912.
46. MICHAEL, A. D. The hypopus question, or the life history of certain Acarina. J. Linn. Soc., Zool. 17 : 371-394. 1884.
47. MILUM, V. G. Larval pests common to nests of bumblebees and comb of the honey-bee. Jour. Econ. Ent. 33 : 81-83. 1940.
48. MITCHELL, T. B. A revision of the genus *Megachile* in the nearctic region. Part I. Classification and descriptions of new species (Hymenoptera: Megachilidae). Trans. Amer. Ent. Soc. 59 : 295-361. 1934.
49. MITCHELL, T. B. A revision of the genus *Megachile* in the nearctic region. Part III. Taxonomy of subgenera *Anthemois* and *Delomegachile* (Hymenoptera: Megachilidae). Trans. Amer. Ent. Soc. 61 : 155-205. 1935.
50. MITCHELL, T. B. A revision of the genus *Megachile* in the nearctic region. Part IV. Taxonomy of subgenera *Xanthosarus*, *Phaenosarus*, *Megachiloides* and *Derotropis* (Hymenoptera: Megachilidae). Trans. Amer. Ent. Soc. 62 : 117-166. 1936.
51. MITCHELL, T. B. Notes and descriptions of nearctic *Megachile* (Hymenoptera, Megachilidae). Pan-Pacific Ent. 18 : 115-118. 1942.
52. MOCSARY, A. Monographia Chrysidarum orbis terrarum universi. Budapest. 1889.
53. MORLEY, C. Ichneumonologia Britannica. I-V. Brit. Mus., London. 1903-1914.
54. MORRISON, F. O. Observations on the numbers and species of bumble bees visiting red clover. Ont. Ent. Soc. Ann. Rpt. 37 : 16-20. 1943.
55. NEAVE, F. The Bremidae of Manitoba. Can. Jour. Res. 8 : 62-72. 1933.
56. NICHOLSON, A. J. The balance of animal populations. Jour. Animal Ecol. 2 (suppl.) : 132-179. 1933.
57. PACKARD, A. S., JR. The humble bees of New England and their parasites; with notices of a new species of *Anthophorabia*, and a new genus of Proctotrupidae. Communic. Essex Instit. 4 : 107-140. Salem, Mass. 1866.
58. PEARSON, J. F. W. Studies in the ecological relations of bees in the Chicago region. Ecol. Monogr. 3 : 373-441. 1933.
59. PIPER, C. V., M. W. EVANS, R. MCKEE, and W. J. MORSE. Alfalfa seed production; pollination studies. U.S. Dept. Agric. Bull. 75. 1914.
60. PLATH, O. E. Bumble bees and their ways. Macmillan & Co. Ltd., London. 1934.

61. PUTNAM, F. W. Notes on the leaf-cutting bee. Communic. Essex Instit. 4 : 105-107. Salem, Mass. 1866.
62. RAU, P. A population study of a bumble bee colony, *Bombus americanorum* F. (Hymen. : Bombidae). Ent. News 52 : 70-73. 1941.
63. ROBERTSON, C. Revised list of oligolectic bees. Ecology. 7 : 378-380. 1926.
64. ROBERTSON, C. Phenology of inquiline and nest-making bees. Psyche 33 : 116-120. 1926.
65. SALT, R. W. Utilize wild bees in alfalfa seed production. Can. Dept. Agr., Field Crops Insect Investig. Mim. Circ. 266. 1940.
66. SLADEN, F. W. L. The bumble bee; its life history and how to domesticate it, with descriptions of all the British species of *Bombus* and *Psithyrus*. Macmillan and Co. Ltd., London. 1912.
67. SLADEN, F. W. L. Characters separating the species of the bee genus *Coelioxys* occurring in Ontario. Can. Ent. 47 : 205-208. 1915.
68. SLADEN, F. W. L. Pollination of alfalfa by bees of the genus *Megachile*. Table of Canadian species of the *latimanus* group. Can. Ent. 50 : 301-304. 1918.
69. SLADEN, F. W. L. Notes on the Canadian representatives of British species of bees. Can. Ent. 51 : 124-130. 1919.
70. SMITH, H. S. The role of biotic factors in the determination of population densities. Jour. Econ. Ent. 28 : 873-898. 1935.
71. THOMPSON, W. R. A catalogue of the parasites and predators of insect pests, section 1, part 4. Parasites of the Hymenoptera, Isopoda and Isoptera. Imperial Parasite Service, Belleville, Canada. 1944.
72. TYSDAL, H. M. Is tripping necessary for seed setting in alfalfa? Jour. Amer. Soc. Agron. 32 : 570-585. 1940.
73. VIERECK, H. L. Guide to the Insects of Connecticut. Part III. Hymenoptera, or wasp-like insects of Connecticut. Apoidea. Conn. St. Geol. Nat. Hist. Surv. Bull. 22. 1916.